THE UNIVERSITY
of EDINBURGH

Evolutionary genetics of immunity to helminths in wild Soay sheep

Alexandra M Sparks

Thesis submitted for the degree of Doctor of Philosophy

School of Biological Sciences

University of Edinburgh

2017

Declaration

The work described in this thesis has been carried out by myself with guidance from my supervisors, unless otherwise stated and detailed below. I have composed this thesis and this work has not been submitted for any other degree or professional qualification.

Chapter 2 and 3 – These chapters use data and plasma samples already collected by others as part of the ongoing St Kilda Soay sheep project. Genomic data was collected and provided by Josephine Pemberton, from which the pedigree was created by Jisca Huisman and Camillo Bérénos. The genomic data underwent quality control and was made available in a GenABEL analysis format for genome wide association studies by Susan Johnston. The antibody assays were carried out by myself, Kathryn Watt and Rona Sinclair with some immune reagents provided by Tom McNeilly. I carried out the statistical analysis and wrote the chapters.

Chapter 4 – This chapter also uses data and plasma samples already collected as part of the Soay sheep project with pedigree data as previously described. The antibody assays were carried out by Michael Evans and Kathryn Watt with some immune reagents provided by Tom McNeilly. I carried out the statistical analysis and wrote the chapter.

Chapter 5 – This chapter also uses data and plasma samples already collected as part of the Soay sheep project with pedigree data as previously described. The antibody assays were carried out by myself and Kathryn Watt with some immune reagents provided by Tom McNeilly. I carried out the statistical analysis and wrote the chapter.

Alexandra M Sparks

Abstract

Parasites have a major impact on host condition and fitness and thereby represent a strong selective force for individuals in wild populations. The main defence against parasite infection and associated morbidity is the host immune response, and consequently it is expected for there to be strong selection eroding genetic variation underlying immune responses in natural populations. However, studies in the wild have found considerable heritable variation underlying immune responses. Few studies have investigated the genetic variants underlying immunity in wild populations and are able to examine how genetic variation is maintained in the face of natural selection. The aim of this thesis is to investigate the selection on, and genetic variation underlying, immunity in a wild Soay sheep population by looking at antibody responses to the prevalent parasite *Teladorsagia circumcincta*. Anti-*T. circumcincta* antibody levels (IgA, IgE, IgG) were measured in neonatal plasma samples taken soon after birth, representing maternally-derived antibodies, and in samples from August yearly from four month old lambs and adults, representing endogenous antibodies. All three endogenously produced antibody measures in lambs and adults were repeatable and heritable. In addition, a genome wide association study run on the three antibody traits on August lamb and adult measures found associations between anti-*T. circumcincta* IgA levels and single nucleotide polymorphisms in a region on chromosome 24. There was evidence for age- and isotype- dependent negative associations between antibody isotypes and strongyle faecal egg counts (FEC). Further, there was evidence for age-dependent selection via positive associations between anti-*T. circumcincta* IgG and survival in females and annual fecundity in males. In comparison, there was no additive genetic variance underlying maternally-derived (neonatal) anti-*T. circumcincta* antibody levels in neonates, but maternal and maternal genetic effects explained a considerable proportion of the variance in these traits. There was evidence for associations between neonatal anti-*T. circumcincta* IgG and later offspring phenotype and fitness, independent of total antibody (IgG) transferred. We found that neonatal anti-*T. circumcincta* IgG levels positively predicted survival to four months old, as well as weight in August. In addition, neonatal anti-*T. circumcincta* IgG levels were associated with reduced strongyle FEC in August, and were associated with improved survival over the first winter. In early life, maternally-derived anti-helminth antibodies are important for early growth, survival, and parasite resistance, as well as first winter survival, while fitness benefits in adulthood were associated with higher endogenous anti-helminth antibody levels. This thesis illustrates that maternal effects and genetic

variation can have strong effects on variation in immunity in the wild, and this variation in turn can have health and fitness consequences for individuals.

Lay summary

Parasites can have strong negative effects on host health, survival and reproduction. Immune responses to parasites are important in determining whether an individual gets infected and, if established, how much disease the infection can cause. Therefore, if immune responses are in part genetically determined, it is expected for there to be selection for individuals with protective immune responses and a reduction in genetic variation underlying these traits. However, studies in wild populations of animals have found considerable genetic variation underlying immune responses. Few studies have investigated the genetic basis of variation in immune responses in wild populations and are able to examine how genetic variation is maintained in the face of natural selection. The aim of this thesis is to investigate the causes and consequences of variation in immunity in a wild Soay sheep population by looking at immune responses (antibodies) to a prevalent parasite (*Teladorsagia circumcincta*). Anti-parasite antibody levels were measured in neonatal plasma samples taken soon after birth, representing antibodies transferred from the mother, and in samples caught in August yearly from four month old lambs, representing antibodies produced by the individual (endogenous). Endogenous anti-parasite antibody levels in lambs and adults were consistent across an individual's lifetime and there was a considerable genetic basis to this variation between individuals. In addition, genetic variants in a region on chromosome 24 were associated with anti-parasite antibody levels of one type (IgA). There was evidence that these anti-parasite antibody levels were associated with lower parasite burdens. In addition, female adults that had higher anti-parasite antibody levels were more likely to survive the winter while male adults with higher anti-parasite antibody levels were more likely to sire more lambs in the following year. In comparison, variation in maternally-derived anti-parasite antibody levels in neonates was associated with genetic variation of mothers rather than offspring. There was evidence for associations between maternally-derived anti-parasite antibody levels and offspring health and survival, independent of total antibody transferred. We found that neonates with higher maternally-derived anti-parasite antibodies were more likely to survive to four months, and were heavier at four months old. In addition, neonates with higher anti-parasite antibody levels had lower parasite burdens at four months old and were more likely to survive their first winter due to this effect on parasite burden. In early life, maternally-derived anti-parasite antibodies are important for early growth, survival and parasite resistance, as well as overwinter survival, while in adulthood higher endogenous anti-parasite antibody levels were associated with higher survival over-winter and improved fecundity in females and males respectively. This thesis illustrates that there is a strong

maternal and genetic basis of variation in immunity in wild populations, and this variation in turn can have health and fitness consequences for individuals.

Acknowledgements

I cannot thank my supervisor, Dan Nussey, enough for taking me on as a PhD student and letting me loose on two incredible datasets. Dan is a fantastic scientist and an endlessly enthusiastic, patient and understanding supervisor. I'm so grateful to have been able to work in such a wonderful lab, and so much of that comes down to Dan – from his dedication in helping others to succeed, right through to pretending to laugh at my terrible jokes. This thesis would not have been possible without his support and encouragement. I'd also like to thank my second supervisor Susan Johnston. I was initially apprehensive of going into the genomic side of this PhD, but I'm so glad I did because it turns out I love running a cheeky GWAS and also because it meant I got to work with Susie. Susie, thank you for being so supportive, for always having time for a chat and for putting up with my panicky emails at all times of the day. Working with Susie has definitely made my thesis infinitely cooler! My unofficial supervisor, Tom McNeilly, has always been so enthusiastic about my work, provided incredibly valuable input and never flinched when I asked a really stupid immunology question. I would also like to thank the following people who have all commented on and improved my work - Rose Zamoyska, Josephine Pemberton, Kathryn Watt, Craig Walling, Rick Maizels, Adam Hayward, Andrea Graham and Jon Slate – and whose encouragement has helped me along the way.

The scope of this project would not have been possible without the incredible long-term Soay sheep dataset and the work of Josephine Pemberton and Jill Pilkington in collecting all the data and plasma samples over the years. I'd like to thank everyone who has helped on this project over the years and especially to those who collected data that formed the foundation of my work. It truly is a fantastic study system and I'm so glad that I got to be a part of it and meet the majestic 4-legged stars of the show.

I'd like to thank all the wonderful members of the Nussey lab for welcoming me into the group and for all their support along the way. I'd like to thank my office - Kathryn, Jen, Luise, Sarah and Rach - for always being there with hugs, advice and at one point a never ending supply of Easter chocolate. Thanks also go to Hannah who I think I hassled for stats and R advice whenever she stepped into our office. I also want to thank Kathryn and Rona for all their hard work in the lab to generate the beautiful datasets I used in this thesis – there is no team I'd rather run hundreds of ELISA plates with. I feel particularly honoured to have witnessed Kathryn get a perfect correlation on an ELISA plate!

I’ve been lucky enough to be part of an incredible PhD cohort. In particular I'd like to thank Amy, Charlotte, Josh, Kara, Kim, Lisa, Mel & Sharon for being with me through the highs and lows of the last four years. I’m so proud of you all and I’m not sure I would have got this far without you. Thank you for all the support and laughs over the years, for putting up with my ‘chicken’ worry sound and my annoying tendency to get songs stuck in people’s heads. Thank you for taking me for my first steak, teaching me how to pronounce tupperware and also providing guidance on what to do with my arms when I finally upgraded to an IEB backpack. You have also saved me on many occasions, including picking up a re-printed BES poster when the original fell out of the poster tube and rolled under a bus when I was on my way to a wedding (Thank you, Kara!). Charlotte may have genuinely saved my life twice. First, saving me from drowning on St Kilda and second in Québec on that canoeing trip turned disaster when I was laughing too hard to paddle. Incidentally, these are also the two funniest moments of my PhD which I still get the giggles about even now.

I have had a number of lovely housemates at West Preston Street who have made my time in Edinburgh so great. I'd especially like to thank my long-term housemates – Lisa and Aoife. Out of all the flatshares in all of Edinburgh I’m so glad I found you both! Thank you for being the urban family I always wanted and for making the flat a home.

To my real family, who have always encouraged and supported me in everything I’ve done, thank you. You’ve always been there for me and the Sparks family sense of humour has got me through many a tough time. Thank you for all the care packages sent both to work and my field sites (although not all turned up!) and for reminding me that there is more to life than a PhD. I realise it has been difficult watching me work incredibly hard for three back to back degrees, I promise this is my last one (probably!).

Last but not least I'd like to thank my best friend - Chris. For always making me laugh, swapping lunches with me when I make inappropriate sandwich choices, reminding me to take breaks and for championing even small progress at the end when my brain was fried. Most importantly, thank you for believing in me when I couldn't believe in myself.

Contents

Chapter 1
General Introduction

1.1 Parasitic helminths

1.1.1 The significance of helminth parasites

Estimates suggest that parasites account for one third to over half of all living species, with parasitic helminth species outnumbering their vertebrate hosts by 50% (Poulin & Morand 2004; Dobson *et al.* 2008; Poulin 2014). Parasitic helminths are worm-like invertebrates that can be classified into three groups; flukes (trematodes), tapeworms (cestodes) and roundworms (nematodes) (Schmid-Hempel 2011). Parasitic helminths are an ancient affliction of humans that have been associated with shaping human history as well as driving human genetic variation (Cox 2002; Hotez *et al.* 2008; Fumagalli *et al.* 2011). Today over 1.5 billion people worldwide are infected with soil-transmitted helminths alone, of which the majority are from the world's poorest communities (WHO 2017). The primary impact of soil-transmitted helminth infection is through morbidity (poor health or disability caused by an infection) rather than mortality. Morbidity can be measured by disability adjusted life years (DALYs) and the combined effect of all helminth infections equated to the loss of 14.2 million DALYs in 2010 (Murray *et al.* 2012). Gastrointestinal (GI) helminth infections are also the largest health threat for grazing ruminants and the estimated annual cost of GI infections in sheep in Great Britain alone are estimated at £84 million, which is exacerbated by increasing prevalence of anthelmintic resistance (Nieuwhof & Bishop 2005; Jackson & Miller 2006). In the wild, helminth infections have the potential to influence populations dynamics through density-dependent reductions in host fecundity or survival (Anderson & May 1978; May & Anderson 1978). This has been demonstrated in populations of red grouse infected with the gastrointestinal nematode *Trichostrongylus tenuis.* Anthelmintic treatment of red grouse prevented the cyclic population crashes observed in control populations implicating the role of parasites in regulating the population (Hudson, Dobson & Newborn

1998). In a population of wild white-footed mice and deer mice infected with numerous intestinal parasites, anthelmintic treatment reduced the extent of seasonal declines compared to control populations, while food supplementation and anthelmintic treatment completely removed population crashes (Pedersen & Greives 2008). The detrimental impacts that these helminth parasites have on host condition, reproduction and survival is expected to lead to strong selection for parasite resistance in natural populations (Fisher 1930; Schmid-Hempel 2011).

1.1.2 Heterogeneity in parasite burden

In general, distributions of parasitic helminth infections between individuals are highly aggregated with a minority of individuals harbouring the majority of the parasite burden (Anderson & May 1985; Shaw & Dobson 1995). The classical aggregation of parasite burden is indicative of heterogeneities in the host population, in addition to differences in exposure to infective stages (Anderson & Gordon 1982). Even small differences between hosts in susceptibility to parasites can produce aggregated distributions of parasites (Anderson & May 1978). Some aspects of variation in parasite burdens have known causes. For instance, males tend to be more heavily infected than females across a range of host-parasite interactions, and this has been attributed to behavioural, morphology and physiological differences between the sexes (Zuk & McKean 1996). Age is also a common factor associated with helminth parasite burden. Burden is often high in early life following initial exposure and before the immune response has had time to develop and mature (Wilson *et al.* 2002; Simon, Hollander & McMichael 2015). The development of adaptive immunity to infection following repeat infection has been associated with a reduction in helminth parasite establishment, survival and reproduction (Harris & Gause 2011; McRae *et al.* 2015) and is considered to be an important determinant of the declining burdens typically observed from early life to adulthood in humans, domesticated ruminants and laboratory animals (Anderson & May 1985; Crombie & Anderson 1985; Woolhouse 1998; McRae *et al.* 2015).

Longitudinal human studies increasingly point to individually distinct and repeatable immune phenotypes, although these studies typically only follow subjects over a small proportion of the adult lifespan (Liston, Carr & Linterman 2016). These individual

differences in the behaviour of the immune response are thought to play an important role in the heterogeneity observed in helminth parasite burden (Maizels & Yazdanbakhsh 2003). There is plenty of evidence that host genetics may underpin this individual level variability: indices of host helminth parasite burden have been found to be significantly heritable in humans (Pullan *et al.* 2010), domestic animals (Bishop & Stear 2001; Riggio *et al.* 2013) and wild populations (Coltman *et al.* 2001a; Beraldi *et al.* 2007). Furthermore, a growing number of studies in humans and domestic animals have identified individual genes or genomic regions associated with variation in helminth burden (Quinnell 2003; Benavides, Sonstegard & Van Tassell 2016). However, much less is currently known about genes associated with immunity to infection in wild mammals.

1.2 Immunity to helminths

The immune system has evolved to defend organisms against a diverse range of pathogens (organisms that cause disease), from viruses to multicellular helminths. The action of the immune system is focussed on recognition of pathogens followed by orchestrating an appropriate response to eliminate or neutralise them. Crucially, the immune system must be able to distinguish between self and non-self antigens, and regulate immune responses to non-pathogenic antigens to prevent conditions such as allergy and autoimmune disease (Alberts *et al.* 2015). The immune response in vertebrates is characterised by two systems: innate immunity and adaptive immunity. Innate immune responses are the first line of defence against pathogens using pre-deployed mechanisms which are able to discriminate between groups of pathogens. In contrast, the adaptive immune response is highly specific to a given pathogen due to antigen-specific lymphocytes, and results in long-term immunological memory (Murphy 2012).

1.2.1 Innate immunity

Following infection with a new pathogen the activation and proliferation of antigen-specific lymphocytes required for the adaptive immune response can take days, during which time the innate immune response is crucial in controlling infections. The innate immune response relies on a limited number of receptors known as pattern recognition receptors (PRRs) which

include membrane bound receptors (e.g. toll-like receptors) as well as soluble receptors in the blood (e.g. complement). These receptors recognise conserved molecular patterns associated with pathogens and the activation of PRRs can result in phagocytosis by cells such as neutrophils and macrophages, the promotion of an inflammatory response, or direct killing by complement (Alberts *et al.* 2015). Complement is a group of plasma proteins that, through a proteolytic cascade, results in the coating of microbial surfaces with complement fragments that promote phagocytosis, inflammation and can directly disrupt microbial cell membranes (Murphy 2012). Phagocytic cells, such as macrophages and neutrophils, are an important component in the innate immune response. Macrophages are long-lived cells found in tissues throughout the body while neutrophils are short-lived cells that are present in blood and are recruited to infection sites in tissues by molecules released by activated macrophages, complement or microbes themselves. For multicellular helminths which are too large to be phagocytised, these cells, together with eosinophils, will surround the parasite and secrete a number of destructive agents such as defensins and toxic products of the respiratory burst to damage the pathogen (Alberts *et al.* 2015). Chemokines and cytokines released by phagocytic cells also promote inflammatory responses that help to fight infection. Inflammation is associated with dilation and increased permeability of blood vessels, increasing blood flow and delivery of immune cells to infection sites (such as neutrophils and monocytes) as well as the flow of lymph containing antigen and antigen-bearing cells into lymphoid tissue (Murphy 2012). These antigen-bearing cells include dendritic cells which form part of the innate immune system but are crucial in the activation and orchestration of the following adaptive immune response (Alberts *et al.* 2015).

1.2.2 Adaptive immunity

The adaptive immune response depends on lymphocytes, specifically B lymphocytes (B cells) and T lymphocytes (T cells), and can lead to immunological memory that can provide long-lasting protection against pathogens. The highly specific nature of adaptive immune responses are due to the unique genetic mechanism that creates receptors on B and T cells that can bind to an almost limitless array of antigens. Activation of B cells is associated with antigen recognition and antibody production, while T cell activation requires recognition of antigens bound to MHC proteins on antigen-presenting cells and leads to further orchestration of an appropriate immune response (Alberts *et al.* 2015).

Adaptive immune responses are initiated by dendritic cells whose function is to carry antigens to lymphoid organs to present and activate T lymphocytes. Immature dendritic cells are present in peripheral tissues and have PRRs to common antigens, which when bound, results in the phagocytosis of microbes. In addition, these cells also continually take up material in the environment, which may contain pathogens or their antigens, by macropinocytosis. After ingestion of pathogens and their products, antigens are displayed as peptides bound to an MHC molecule. Following recognition of pathogens, dendritic cells migrate via the lymphatic system to regional lymph nodes. On arrival at the lymph node as mature dendritic cells, also known as antigen-presenting cells (APCs), they express both the antigen and co-stimulatory molecules needed to activate antigen-specific naïve T lymphocytes (Murphy 2012). Each naïve T lymphocyte bears antigen receptors of a single specificity, and the binding of antigens displayed by APCs causes these cells to proliferate and differentiate into effector cells bearing receptors of identical specificity (Alberts *et al.* 2015). There are several functional types of effector T cells. Cytotoxic T lymphocytes are involved with direct killing of infected cells. Helper T cells are important in activating B cells and other immune cells and orchestrating the immune response. There are a number of subsets of T helper cells, of which the first to be identified were T_H1 and T_H2 subsets, which produce different cytokines and have different effector functions. T_H1 cells are typically associated with microbial infections, produce the cytokine interferon gamma (IFNγ) and are associated with activating macrophages to improve microbial-killing activity. In contrast, T_H2 cells are associated with helminth infections and produce T_H2 cytokines (IL4, IL5, IL13) which are important in activating eosinophils, mast cells and basophils. Further, T_H2 cells are crucial in class switching of B cells to produce IgE antibodies which are associated with protection against parasites. Finally, regulatory T cells (T_{reg}) are involved in suppressing and regulating the immune response (Murphy 2012).

Effector T helper cells have an important role in activating B cells and orchestrating the humoral or antibody-mediated immune response. Following binding of an antigen to the B cell receptor on B cells, this antigen is internalised and returned to the surface as a peptide-MHC complex. In addition to recognition of antigens by B cells, a second signal is needed and typically received by activated helper T cells which recognise the peptide-MHC complex. Following the activation of B cells, they proliferate and differentiate into effector cells known as plasma cells which secrete antibodies (Murphy 2012). Antibodies are the secreted form of the B cell receptor and possess the same unique antigen-binding site and are

the major effector response of humoral immunity (Alberts *et al.* 2015). However, these antibodies (or immunoglobulins) have a limited life-span and following removal of the antigen, most B and T cells undergo apoptosis. Long-lived memory B and T cells formed as part of the adaptive immune response are the basis of immunological memory and provide lasting immunity by providing a quicker and more efficient response on subsequent encounter with the same pathogen (Murphy 2012).

Antibodies are made up of two identical heavy and light chains which both have variable and constant regions. The variable region of the light and heavy chain combine to form the antigen-binding site, which can bind to a diverse variety of antigens. The constant or Fc region determines which molecules the antibody can bind to and their subsequent effector functions (Alberts *et al.* 2015). Antibodies have a range of effector functions, including neutralising toxins, opsonising pathogens to make them more readily ingested by phagocytic cells, sensitisation of mast cells or activation of complement. Mammals have five classes (or isotypes) of antibodies: IgA, IgE, IgD, IgG and IgM. Each isotype has a different class of heavy chain. IgM can be produced before class-switching by immature naïve B cells to other isotypes and are the major class of antibody secreted into the blood following first exposure to an antigen. IgM antibodies are typically of lower affinity than other classes. To compensate, IgM antibodies form pentamers, making them effective activators of the complement system (Murphy 2012). Following migration from the bone marrow, B cells also begin to produce cell-surface IgD molecules, however, these IgD molecules tend not to be secreted (Alberts *et al.* 2015).

In response to antigen stimulation or costimulatory signals, B cells can undergo class switching to produce IgA, IgE and IgG antibodies that can more easily diffuse into tissues (Alberts *et al.* 2015). IgG is the most common antibody isotype in blood and extracellular fluid, and is effective at neutralising toxins and activating complement. Additionally, IgG acts as an opsonin, coating pathogens and marking them for uptake by phagocytic cells. IgA is the most common antibody isotype in secretions and at mucosal surfaces, and acts as a strong neutralising antibody, but compared to IgG is a weak opsonin and activator of complement. IgA is principally secreted by plasma cells in the lamina propria below epithelial cells. Dimeric IgA antibodies are then transported to the gut lumen via the polymeric immunoglobulin receptor (pIgR) by transcytosis. In contrast, there are very low levels of IgE antibodies in circulation. IgE is produced by plasma cells in lymph node

draining areas of antigen entry and by plasma cells at the site of infection, typically in the mucosal tissue or skin (Murphy 2012). Further, IgE has the shortest half-life of any antibody isotype (Wu & Zarrin 2014), however, it is typically found bound to mast cells via their Fc receptor and has a longer half-life in this form (Negrão-Corrêa 2001). In the gut, immune cells are primed to produce a T_H2 response to parasitic infection and these T_H2 cells secrete cytokines such as IL4 and IL13 which signal B cells to switch to IgE production (Murphy 2012). Mast cell degranulation, mediated by antigen binding to IgE, releases chemical mediators that promote allergic inflammation. In addition, IgE molecules also bind to Fc receptors on basophils and eosinophils which also release a variety of cytotoxic molecules (Alberts *et al.* 2015).

1.2.3 Immunity to helminth parasites in mice & humans

The immune system faces a variety of challenges in preventing the establishment of larvae and removing adult helminth parasites from the body. Helminths are long-lived, are much larger than human cells while nematodes in addition have a resistant cuticle. These parasites can be found virtually anywhere in the body and have a diverse variety of life cycles (Maruyama & Nawa 2002). In general, protective immunity to helminth parasites is associated with a T_H2 response, although the effector mechanisms may differ between parasitic species and life stages (Harris & Gause 2011). T_H2 responses are typically associated with an increase in levels of T_H2 cytokines (such as IL4, IL5, IL13), CD4+ T_H2 cells, eosinophils, mast cells, basophils, alternatively activated macrophages and plasma cells secreting IgE antibodies (Allen & Maizels 2011). Studies of human helminth infections (*Brugia malayi* and *Schistosoma mansoni*) have typically found that individuals with a pro-inflammatory T_H1 response have clinical disease, individuals with a T_H2 response have asymptomatic infections, while those with a balanced response due to T_{reg} subsets are most likely to be resistant to infection (Maizels & Yazdanbakhsh 2003). However, it may not always be the case that immune responses are able to fully clear helminth infections without significant collateral tissue damage, in which case immune responses may be down-regulated and parasites tolerated during later infection (Allen & Maizels 2011).

Studies in laboratory mice, particularly those using experimental infections with *Heligmosomoides polygyrus,* provide an important foundation for our understanding of the

development and maintenance of mammalian immunity to gut helminths. Infection with *H. polygyrus* occurs following ingestion of the infective L3 larval stage, which then migrate to the small intestine and invade the submucosa. After developing into adult worms, they migrate back to the intestinal lumen where males and females mate to produce eggs (Anthony *et al.* 2007). Primary infection to *H. polygyrus* in mice is chronic and associated with adult parasites in the gut. Following elimination by helminth-specific drugs, secondary infection triggers a T_H2 response which results in worm expulsion within 14 days (Gause, Urban & Stadecker 2003). During secondary infection, a large number of alternatively activated macrophages are present around invading larvae, in addition to neutrophils, dendritic cells, eosinophils, CD4+ T cells and levels of T_H2 cytokines. In addition to promoting parasite clearance, T_H2 responses are also important in downregulating pro-inflammatory T_H1 responses and healing damaged tissue typically by the actions of alternatively activated macrophages and eosinophils. In addition, expulsion of live parasites is promoted by an increase in luminal fluids and muscle contractility (Anthony *et al.* 2007).

Murine models have been crucial in identifying the role of B cells and antibodies in protection against helminth infections. Studies using B cell deficient mice have shown that protective immunity to challenge *H. polygyrus, Litomosoides sigmodontis* and *Trichuris muris* is B cell dependent while expulsion of *Nippostrongylus brasiliensis* helminths is CD4+ T cell dependent and does not require B cells (Blackwell & Else 2001; Martin *et al.* 2001; McCoy *et al.* 2008; Wojciechowski *et al.* 2009; Liu *et al.* 2010). In addition, isotype-switched or affinity-matured antibodies were needed for protective immunity to *H. polygyrus* infection (McCoy *et al.* 2008; Wojciechowski *et al.* 2009). The importance of IgG has been particularly implicated in protective immunity to infection in these models, with passive immunity by purified IgG from immune serum demonstrated in *T. muris* and *H. polygyrus* infection (Blackwell & Else 2001; McCoy *et al.* 2008) and maternal antibody transfer of parasite-specific IgG associated with protection of neonates to *T. spiralis* and *H. polygyrus* infection (Appleton & McGregor 1987; Harris *et al.* 2006). There is some evidence that IgA is also somewhat important in *H. polygyrus* (McCoy *et al.* 2008) and IgM antibodies are important in filarial infections (Rajan, Ramalingam & Rajan 2005). In contrast, despite the high levels of IgE antibodies observed in helminth infection, this antibody isotype was not required for protective immunity to *H. polygyrus* (McCoy *et al.* 2008), *Nippostrongylus brasiliensis* (Watanabe *et al.* 1988) or *Schistosoma mansoni* (El Ridi, Ozaki & Kamiya 1998). In *H. polygyrus* infection it is thought that antibodies can affect worm migration and

development (Liu *et al.* 2010), and by activating macrophages lead to trapping of larvae and promotion of wound healing (Esser-von Bieren *et al.* 2013). Polyclonal IgG had no effect on the number of larvae developing into adults but did reduce female worm fecundity (McCoy *et al.* 2008). However, the protective role of antibodies in helminth infection appears to be parasite-specific, as some studies have noted that the transfer of protective antibodies via immune serum or monoclonal antibodies have not induced a protective effect (Harris & Gause 2011).

1.2.4 Immunity to helminth parasites in domestic sheep

Due to the important negative effects of gastro-intestinal nematode (GIN) parasites in the health and productivity of domestic sheep, there is a broad literature and a good understanding of the basis of resistance to infection in major parasite species. One agriculturally significant and widely studied GIN species in the UK is the strongyle species, *Teladorsagia circumcincta*. *T. circumcincta* infection is associated with parasitic gastroenteritis and is a major cause of disease in lambs (McNeilly, Devaney & Matthews 2009). Following repeat exposure of lambs to infection, protective immunity can develop by around 12 months of age. Development of protection against infection with *T. circumcincta*, like many other GIN infections, is associated with a T_H2 response including mast cells, eosinophils, T_H2 cytokines and parasite-specific IgA, IgE and IgG1 levels. With continued exposure to infection, adults retain resistance and typically harbour few adult worms. Development of immunity to *T. circumcincta* is initially associated with immune responses that suppress worm growth while later immune responses are associated with worm killing and expulsion (McRae *et al.* 2015).

The focus on antibody responses in *T. circumcincta* infection has typically been on IgA and IgE as they are thought to be the main players at mucosal surfaces. In lambs, reduced nematode faecal egg counts (FEC) are associated with increased parasite-specific IgA levels directed at worm growth and subsequent female fecundity (Stear *et al.* 1995; Strain *et al.* 2002). IgA responses have also been associated with the arrestment of development of L4 larvae (Stear *et al.* 2004). Anti-nematode IgE levels are also negatively associated with FEC (Huntley *et al.* 2001). In sheep that have developed resistance to infection, a hypersensitive reaction is expected to lead to expulsion of incoming L3 larvae (the infective larval stage of

the parasite, see Fig 1.4) and the arrest of development of L4 larvae, moderated in part by IgA (McNeilly *et al.* 2009). In addition, IgE-dependent mast cell degranulation is associated with reducing adult worm burden (Stear *et al.* 1995; Huntley *et al.* 2001). Despite the importance of IgG in protective immunity to helminth infections in mice (Appleton & McGregor 1987; Blackwell & Else 2001; Harris *et al.* 2006; McCoy *et al.* 2008), fewer studies have looked at the protective effect of IgG in *T. circumcincta* infection. Those that have have documented negative associations between parasite-specific IgG levels and FEC or worm burden in adults (Williams *et al.* 2010; McBean *et al.* 2016) while IgG has been implicated indirectly by associations between complement and protective immune responses in other ruminant-worm interactions (Li *et al.* 2010; Guo *et al.* 2016). In addition, there is evidence that IgG is associated with resistance to strongyles (Hayward *et al.* 2014) and is an important biomarker of health in wild adult Soay sheep (Nussey *et al.* 2014; Watson *et al.* 2016). However, the focus in ovine studies have typically been lambs and the development of immunity, due to the interest in selective breeding (Atlija *et al.* 2016; Benavides *et al.* 2016). As a result, less is known about how immunity is maintained and whether tolerance (the ability of a host to limit the damage caused by a given parasite burden (Råberg, Graham & Read 2009)) is important in older age groups. This is particularly important since ewes can contribute massively to larval pasture counts during the peri-parturient rise where immunity to gastrointestinal nematodes is relaxed (McRae *et al.* 2015).

1.3 Studying immunology in the wild

An effective immune response to parasites is critical in determining susceptibility to infection, disease progression and associated pathology (Anthony *et al.* 2007; Allen & Maizels 2011). This is especially true in wild populations who face constant challenge with a multitude of diverse micro- and macroparasites (Lazzaro & Little 2009). Our current knowledge of mammalian immune responses and resistance to parasites have largely been determined from studies of inbred laboratory rodents under artificial conditions (Maizels & Nussey 2013). However, the immune system of the common laboratory mouse is immature compared to pet store or wild mice and reflects the immune status of human neonates rather than adults (Beura *et al.* 2016). As a result, studies from laboratory mice are unlikely to translate to their wild counterparts, whom are more genetically diverse, experience a wider range of environmental conditions, may suffer from multiple infections and be of a variable

nutritional status (Pedersen & Babayan 2011). Wild mice have been documented to have improved immune function, with higher concentrations of a number of antibodies and as well as greater overall activation of immune cells (Abolins *et al.* 2011, 2017). Notably, studies in the wild have documented that the immune response is far more variable than expected from these laboratory studies (Pedersen & Babayan 2011).

The field of eco-immunology is associated with identifying the underlying causes of variation in immune responses and consequences of this variation under natural conditions. From an ecological and evolutionary perspective, parasites can cause physiological damage and reduce host fitness if hosts are unable to mount an effective immune response (Sheldon & Verhulst 1996). However the activation and maintenance of immune responses is expected to incur considerable energetic costs (Lochmiller & Deerenberg 2000), drawing resources away from other functions such as growth and reproduction (Sheldon & Verhulst 1996; Schmid-Hempel 2003). There is good evidence from wild birds that mounting an immune response has costs for life history traits associated with reproduction (Ilmonen, Taarna & Hasselquist 2000; Bonneaud *et al.* 2003; Marzal *et al.* 2007). However, there is also growing support for links between markers of immune responsiveness and host survival in birds (Saino, Bolzern & Møller 1997; Christe, Møller & de Lope 1998; Christe *et al.* 2001; Merino, Møller & de Lope 2000). However, these studies have predominantly used very general measures of "immunocompetence" such as the phytohaemagglutinin (PHA) response, leucocyte counts and bacterial killing assays. In-depth studies in the wild measuring immune responses directed at ecologically relevant parasites, that link immunity, parasite burden and fitness remain rare (Graham *et al.* 2011; Demas *et al.* 2011). In addition, not all immune responses are expected to increase fitness, and strong immune responses may lead to damage of host tissue (Viney, Riley & Buchanan 2005; Graham, Allen & Read 2005). There is a growing call for field studies that can harness reagents and tools developed in model and domestic animals to measure immune phenotypes in greater detail and to understand the causes and consequences of variation in immune phenotypes under natural conditions (Pedersen & Babayan 2011).

1.4 Quantitative genetics

To understand the evolutionary potential of traits, it is crucial to know how much variation in the trait is due to genes. Quantitative genetics is the study of the genetic basis of quantitative traits that are likely influenced by a large number of genes each having a small effect on the trait. Quantification of the additive genetic variance underlying a trait, and its heritability, can be calculated if the relatedness between individuals is known. The easiest way to do so is by parent-offspring regression or full- or half-sib analyses (Falconer & Mackay 1996). Where comprehensive knowledge about the relatedness of individuals in a population is known through a population pedigree, additive genetic variance underlying a trait can be determined by looking at the covariance between relatives of varying degrees using a form of linear mixed model known as an "animal model". The similarity between a pair of individuals in a given trait is determined by the relatedness of the pair and the degree of genetic variance underlying the trait (Lynch & Walsh 1998). The proportion of phenotypic variance explained by the additive genetic variance (known as the narrow-sense heritability - h^2) can then be compared to the variance explained by other genetic and non-genetic effects (Falconer & Mackay 1996). In wild populations, directional selection on a heritable trait is expected to drive alleles associated with beneficial phenotypes to fixation and remove those with alleles associated with less fit genotypes (Fisher 1930). As a result, genetic variance underlying fitness-related traits is expected to be eroded by directional selection (Falconer & Mackay 1996). However in wild populations considerable genetic variance underlying such traits exist (Postma 2014). Quantitative genetic models can help explain how genetic diversity is maintained in the face of natural selection by revealing trade-offs in terms of genetic conflicts or constraints, that are not apparent at the phenotypic level (Kruuk, Slate & Wilson 2008). In many cases, directional selection has been observed on traits, but stasis or responses are observed in the opposite direction (Merilä, Sheldon & Kruuk 2001). Analyses of long-term data in wild systems have documented how genetic constraints, in addition to fluctuating selection pressures dependent on environment, sex and age may explain these observations (Kruuk 2004; Kruuk *et al.* 2008).

Quantitative genetic animal models also allow other components underlying phenotypic variance to be modelled, including common environment effects, genotype-by-environment, genotype-by-age and maternal effects (Kruuk & Hadfield 2007; Kruuk *et al.* 2008). Variation in phenotypic traits are determined by an individual's genes and the environment

they experience. An important part of this environment is their interactions with conspecifics, and an important interaction is between offspring and their parents (Mousseau & Fox 1998). In mammals in particular, interactions between mother and offspring can have huge impacts on offspring development and fitness, due to the extended period of maternal care during gestation and lactation (Reinhold 2002; Maestripieri & Mateo 2009). The influence of the mother's phenotype on the phenotype of her offspring above and beyond her genetic contributions are known as maternal effects (Mousseau & Fox 1998). Maternal effects are widespread across taxa, and are typically greatest for early life traits, such as birth weight in Soay sheep (Wilson *et al.* 2005) and offspring size in pied flycatchers (Potti & Merino 1994). Maternal effects can have both an environmental and genetic basis (Mousseau & Fox 1998; Wolf *et al.* 1998). Maternal genetic effects, a type of indirect genetic effect, can have consequences on evolutionary dynamics since it provides an additional source of genetic variation on which selection can act. As a result, evolutionary time lags can be observed because the response to selection on the offspring generation will also be dependent on the strength and direction of selection acting on the maternal generation (Kirkpatrick & Lande 1989). Depending on the covariance between the direct additive genetic effect of the offspring's genes and the maternal additive genetic effect, maternal genetic effects can either slow down or accelerate the rate of evolution of a character or can lead to evolution in the opposite direction to selection on the offspring trait (Kirkpatrick & Lande 1989; Wolf *et al.* 1998).

Standard quantitative genetic animal models assume a large number of genes of small effect underlie trait heritability (Falconer & Mackay 1996; Lynch & Walsh 1998). In humans, genome wide association studies (GWAS) have identified hundreds of single nucleotide polymorphisms (SNPs) associated with complex traits (Donnelly 2008; Hindorff *et al.* 2009) but SNPs only explain a small proportion of the heritability of these traits leading to the 'missing heritability' problem (Maher 2008; Manolio *et al.* 2009). This may be due to SNPs with small effects not meeting threshold significance, and in support of this a study combining the effects of common SNPs found that in combination these SNPs explained 56% of the heritability in human height (Yang *et al.* 2010). With the accumulation of affordable genomic resources for non-model species, GWAS in wild populations are beginning to emerge (Slate *et al.* 2010; Ellegren 2014). These have been particularly successful for identifying genomic regions associated with Mendelian traits, such as horn morphology in Soay sheep (Johnston *et al.* 2011) and age at maturity in Atlantic salmon

(Barson *et al.* 2015). However, the small sample sizes in natural populations, in addition to low linkage disequilibrium between SNPs and causal variants and small effect sizes of SNPs underlying polygenic traits means that many of these studies are typically underpowered (Slate *et al.* 2010). As a result, GWAS studies in the wild typically find few, or no associations between SNPs and phenotypic traits (Johnston *et al.* 2014; Bérénos *et al.* 2015; Husby *et al.* 2015; Santure *et al.* 2015; Wenzel *et al.* 2015; Kardos *et al.* 2016; Silva *et al.* 2017). Where associations have been identified, large effect sizes of QTL are reported (Tarka *et al.* 2010; Bérénos *et al.* 2015; Husby *et al.* 2015; Johnston *et al.* 2016). However, small sample sizes may lead to over-estimation of allele effect sizes (known as the Beavis effect) or may lead to false associations between SNPs and phenotypes due to chance, particularly at rare alleles (Beavis 1994; Slate 2013). Further, the traits investigated in wild populations are typically morphological or skeletal traits, and the genetic basis of physiological traits have often been overlooked (Postma 2014).

Immune genes are some of the most variable in the vertebrate genome, and understanding why so much variation is maintained is a key question in evolutionary genetics (Prugnolle *et al.* 2005). Only by studying wild populations, where natural selection is occurring, is it possible to determine how and why so much genetic variation in immune responses is maintained under natural selection (Maizels & Nussey 2013). Studies in the wild have provided mixed responses as to whether immune responses are heritable. In birds, PHA responses have been found to be significantly heritable in some studies (Bonneaud *et al.* 2009; Drobniak *et al.* 2010; Kim *et al.* 2013) but not in others (Pitala *et al.* 2007; Sakaluk *et al.* 2014). However, only a few of these studies have used an animal model approach (Pitala *et al.* 2007; Kim *et al.* 2013; Sakaluk *et al.* 2014). In comparison, parasite-specific pan isotype and anti-nuclear antibody levels have been shown to be heritable in wild Soay sheep (Graham *et al.* 2010; Hayward *et al.* 2014). Studies of the evolutionary genetics of immune responses in the wild are typically restricted by the difficulties in constructing pedigrees for natural populations (Pemberton 2008), in addition to the lack of genomic data and immune assays for non-model species (Bradley & Jackson 2008). As a result, studies identifying genes or genomic regions associated with immunity in the wild have been limited to candidate gene studies typically on major immune genes such as the major histocompatibility complex and cytokine genes (Coltman *et al.* 2001b; Bonneaud *et al.* 2009; Turner *et al.* 2011; Brown *et al.* 2013).

In addition to a genetic basis of immunity, in early life mothers have a huge impact on the development of immunity of offspring via maternal antibody transfer. Maternal antibodies are crucial in providing neonates with humoral immunity during a period where their own immune system is not yet developed. Antibodies can be transferred both prenatally via the placenta and/or postnatally via colostrum, milk or yolk depending on the species. Typically, the duration of passive immunity (the transfer of antibodies or immune serum from an immune individual to a naïve individual conferring protection) coincides with the development of active immunity (the ability of an individual to produce antibodies in response to an antigen) in the offspring (Grindstaff, Brodie & Ketterson 2003). Direct protection against helminth parasites via maternal antibodies has been observed in mice (Appleton & McGregor 1987; Harris *et al.* 2006) and lack of passive immune transfer has been associated with growth retardation and reduced survival of offspring (Gustafsson *et al.* 1994). In domestic livestock, failure of passive transfer of antibodies is associated with reduced growth rates, high neonatal morbidity and mortality rates (Robison, Stott & DeNise 1988; Wittum & Perino 1995). Although it appears that mothers are fairly consistent in the levels of antibodies transferred to offspring in wild birds (Coakley *et al.* 2014), and there is a large maternal genetic effect underlying maternal antibody transfer in pigs (Rohrer *et al.* 2014), no studies have quantified the maternal effects or applied quantitative genetic models to investigate the genetic basis of maternal antibody transfer in the wild (Boulinier & Staszewski 2008). In addition, only a handful of studies have documented growth or survival benefits of maternal antibodies in the wild (Heeb *et al.* 1998; Buechler *et al.* 2002; Kallio *et al.* 2006; Pihlaja, Siitari & Alatalo 2006). It is highly likely that these studies are limited by the availability of immune reagents for non-model species as well as the knowledge of natural parasites and protective immune responses to them (Boulinier & Staszewski 2008).

The aim of this thesis is to investigate the causes of variation in helminth-specific immune responses and the consequences of immune variation for the health and fitness of individuals in the wild.

1.5 Soay sheep on St Kilda

Approximately 160km north-west of the Scottish mainland lies the St Kilda archipelago (Figure 1.1). It consists of four main islands: Soay, Dun, Hirta and Boreray. Human settlement on Hirta, the largest island, was first established in the Bronze Age, but the population gradually fell until permanent residence on the island was considered unfeasible and the community were evacuated in 1930. On the nearby island of Soay, an ancient breed of domestic sheep, Soay sheep, had been living unmanaged for several millennia. Soay sheep are around a third of the size of most domestic sheep breeds and unlike a number of modern sheep breeds are horned. To maintain grazing, 107 sheep were moved from Soay to Hirta in 1932. Since then, the population of Soay sheep on Hirta has been living unmanaged (Clutton-Brock & Pemberton 2004).

The Soay sheep population in the village bay area of Hirta, which comprises around a third of the island's total population, have been the subject of a long-term individual based study since 1985 (Figure 1.1). In the absence of predation and human management, deaths are associated primarily with starvation over winter and occur between January-April. The population dynamics of the Soay sheep are characterised by periods of low but rising population sizes followed by high mortality 'crash' winters in which over half the population can die (Figure 1.2, Clutton-Brock & Pemberton 2004). Contributing to these 'crash' winters are climate, population density and demography (Catchpole *et al.* 2000; Coulson *et al.* 2001, 2008).

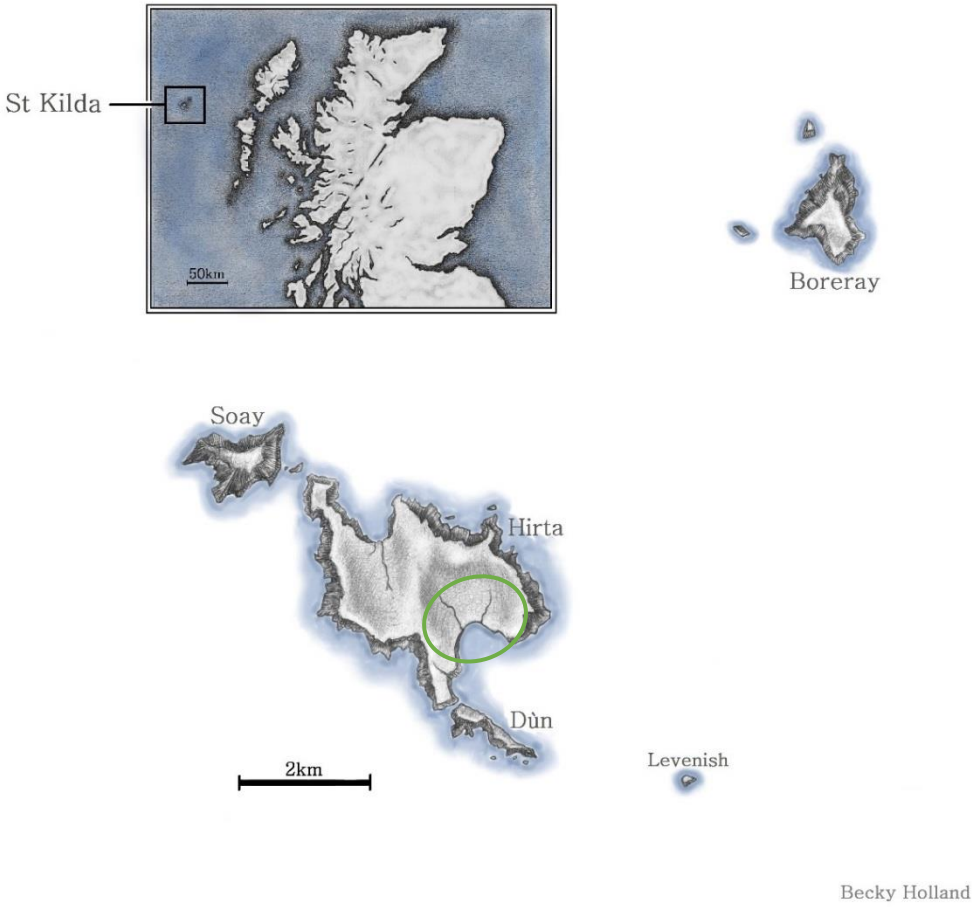

Figure 1.1. The location and islands of the St Kilda archipelago (Map courtesy of Becky Holland). The study population of the Soay sheep project are individuals in the Village Bay area of Hirta (circled), which is shown in the photograph below taken in August 2016.

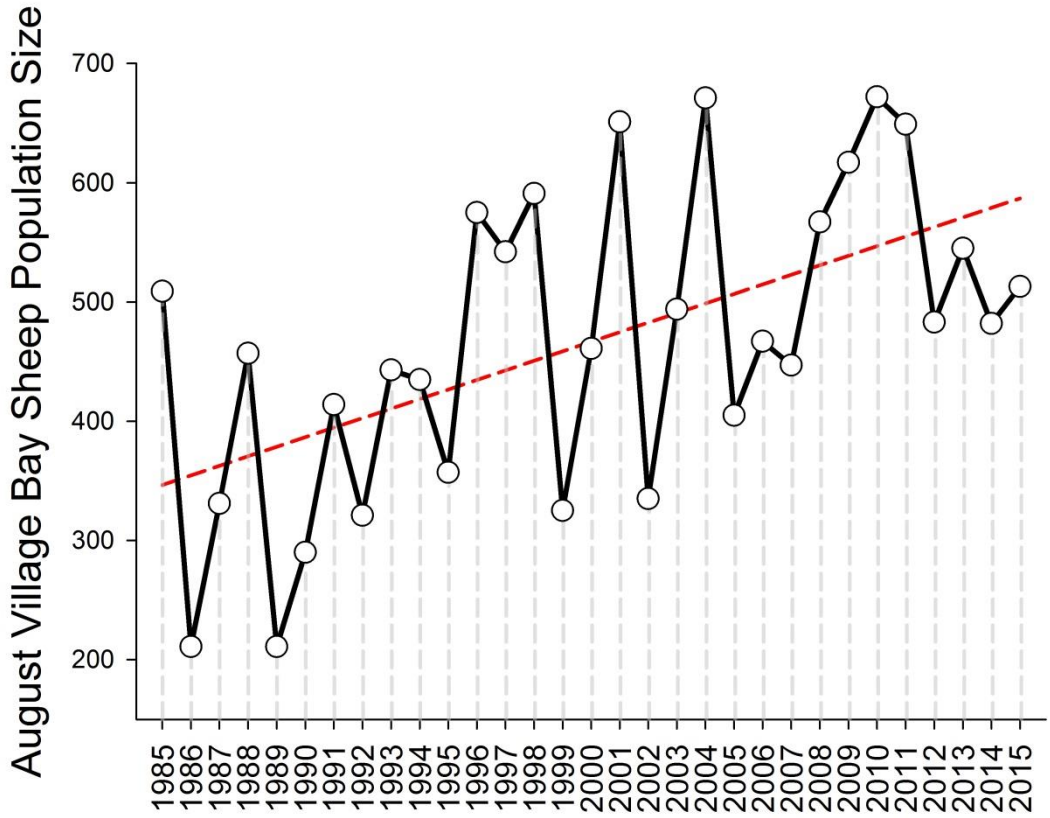

Figure 1.2. Size of the Village Bay population of Soay sheep recorded in August between 1985-2015 (open circles). Population dynamics are highly unstable, and there is a general increase in population size over time (dotted line).

1.5.1 Data collection

Three main expeditions to Hirta occur each year in spring (coinciding with lambing), summer and autumn/winter (coinciding with rutting) (Figure 1.3). In spring, between March-May, fieldworkers visit the island to identify sheep that have died over the winter and to catch and tag lambs. The whole study area is searched for carcasses and ear tags, and the carcasses of 85% of sheep originally tagged as lambs are recovered. Lambing begins around March, and lambs are caught and tagged and the identity of the mother recorded. The ear punch samples from the tagging procedure are used for genetic analyses. Additionally, lambs are weighed and have blood samples taken. Around 95% of all lambs born in the study area are caught each year. In summer, between July-August, members of the project attempt to catch as many sheep (~50%) in the study area as possible using temporary corrals. At

capture, a number of morphometric traits are measured, including: weight, horn length and circumference, hind and fore leg length and scrotal circumference. In addition, blood and faecal samples are taken, females checked for presence of reproductive status and milk and ectoparasites counted. During this same period of data collection, a count of the island population is carried out. The last data collection period occurs between October and November to coincide with rutting activity, and censuses recording reproductive behaviour are performed daily and any unmarked immigrant males are immobilised, blood sampled for genetic analyses and morphometric data collected. During each of the three trips, 10 censuses are carried out (Clutton-Brock & Pemberton 2004).

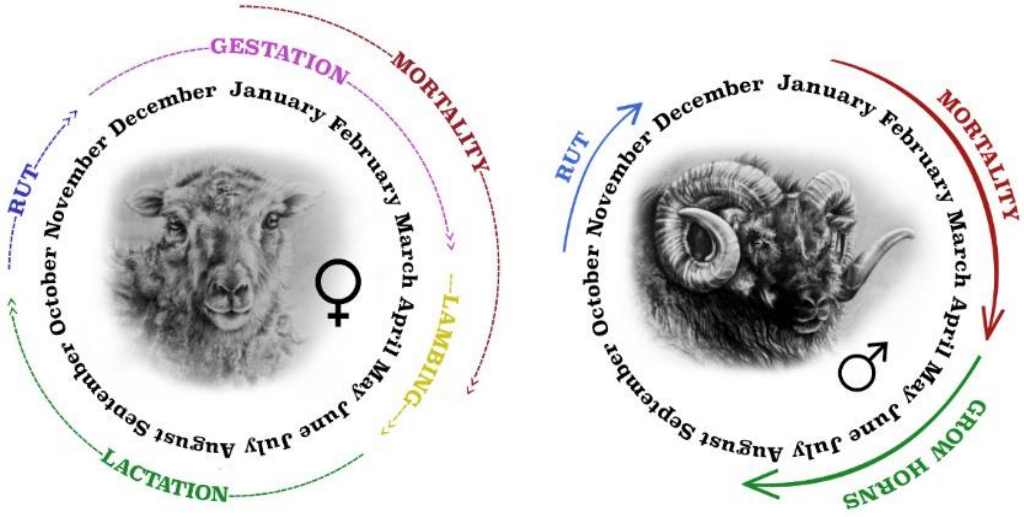

Figure 1.3. Events in the sheep year for females (left) and males (right) including the reproductive cycle and the period of high mortality over-winter (Diagrams courtesy of Becky Holland).

1.5.2 Reproduction and life history

During the rut, males engage in head-butting contests and compete for dominance and access to females. Soay sheep are sexually mature in their first year of life, but male breeding success is typically low for lambs and yearlings and is negatively associated with population density (Clutton-Brock & Pemberton 2004), but positively associated with horn size (Preston *et al.* 2003; Robinson *et al.* 2006). In contrast, prime-aged adult ewes typically have lambs each year. Annual fecundity is lower for female lambs and is dependent on their weight and

population density (Clutton-Brock & Pemberton 2004) and older females where there is evidence of a senescent decline in fecundity (Hayward *et al.* 2013). Between 2-23% of litters each year are twins and the probability of ewes having twins is positively related to their age and weight (Clutton-Brock *et al.* 1991; Clutton-Brock & Pemberton 2004; Hayward *et al.* 2013). The birth weight of lambs are higher from prime-aged, heavier mothers, and are lower for twins (Clutton-Brock *et al.* 1996; Clutton-Brock & Pemberton 2004). Lambs born lighter are less likely to survive the neonatal period (Clutton-Brock *et al.* 1992). By around two weeks old, lambs suckling frequency and duration declines and they are weaned by summer (Robertson *et al.* 1992).

Over-winter survival of sheep is dependent on a complex interaction between food availability, winter weather and the demographic structure of the population (Coulson *et al.* 2001). At high sheep densities, wet and windy winter weather conditions are thought to trigger high mortality 'crash' events, when large numbers of animals die. However, lambs have lower winter survival probabilities than any other demographic group and lamb mortality rates can be high even in non-crash winters. Males also have lower survival rates, regardless of their age (Clutton-Brock & Pemberton 2004). Survival rates are highest from around two years onwards, when animals are in prime age, but there is an age-related decline in annual survival probability from four onwards, and from around seven years onwards sheep are considered geriatric (Nussey *et al.* 2012; Hayward *et al.* 2015). Female Soay sheep live longer than males, and the shorter lifespan of males is linked to reproductive effort since castrates not participating in the rut lived longer than both adult males and females (Clutton-Brock & Pemberton 2004).

1.5.3 Parasitology

Soay sheep are infected with a number of micro- and macroparasites. These include 13 species of protozoa (*Cryptosporidium parvum, Giardia duodenalis* and 11 species of *Eimeria*) (Wilson *et al.* 2004; Craig *et al.* 2007). In addition, 15 helminth parasites have been recorded: two species of tapeworm (*Moniezia expansa, Taenia hydatigena*) as well as 13 species of nematodes including species located in the lung (*Dictyocaulus filarial, Muellerius capillaris*), abomasum (*Teladorsagia circumcincta, Trichostrongylus axei*), small intestine (*Trichostrongylus axei, Trichostrongylus vitrinus, Capillaria longipes, Strongyloides*

papillosus, Nematodirus battus, Nematodirus filicollis, Nematodirus helvetianus, Bunostomum trigonocephalum) and large intestine (*Trichuris ovis* and *Chabertia ovina*) (Wilson *et al.* 2004; Craig, Pilkington & Pemberton 2006).

Strongyle faecal egg counts are carried out using a modified McMaster technique and are comprised of eggs of 5 species that are indistinguishable by eye (*T. circumcincta, T. axei, T. vitrinus, C. ovina* and *B. trigonocephalum*) (Wilson *et al.* 2004). Contributing to the majority of the strongyle FEC burden is *Teladorsagia circumcincta, Trichostrongylus axei* and *Trichostrongylus vitrinus* (Craig *et al.* 2006). In the sheep, *T. circumcincta* infection is expected to be one of the most predominant and pathogenic species (Wilson *et al.* 2004). *T. circumcincta* is also a prevalent parasite of domestic sheep in temperate regions, and is a major cause of parasitic gastroenteritis in grazing sheep in these regions (McNeilly *et al.* 2009). The prevalence of strongyle infection in Soay sheep is high, and there is a positive correlation between strongyle FEC and *T. circumcincta* burden in the abomasum (Gulland & Fox 1992; Grenfell *et al.* 1995). In autopsy samples of dead sheep, most harboured many thousands of worms in their gastrointestinal tract and there was a 100% prevalence of *T. circumcincta* in the abomasum, with burden peaking in two year old sheep (Craig *et al.* 2006).

T. circumcincta and related strongyle parasites have a direct life cycle and hosts become infected by ingesting infective third stage larvae (L3) with vegetation (Figure 1.4). In the gastric glands, they go through 2 more moults, before emerging from the gastric glands as adults and attach themselves to the walls of the abomasum. Adult worms live on average for 50 days. Eggs can appear in the faeces around 18 days post infection, and these eggs can survive on the pasture for many months dependent on climate. Following hatching, larvae go through 2 moults to becoming infective L3 again (Wilson *et al.* 2004).

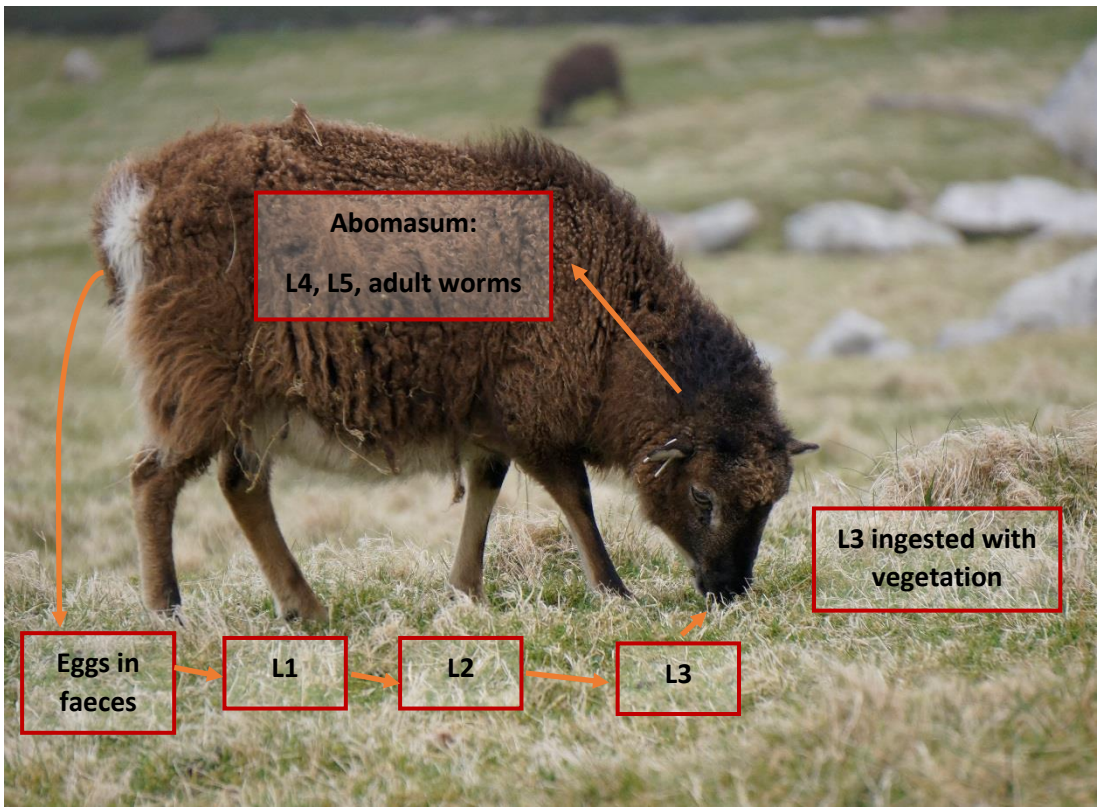

Figure 1.4. Life cycle of *Teladorsagia circumcincta* following (Wilson *et al.* 2004). Photo courtesy of Kara Dicks.

Exposure to parasites may occur at a very young age since Soay lambs have been observed to nibble grass within their first week of life (Robertson *et al.* 1992; Clutton-Brock & Pemberton 2004). Lamb strongyle FEC has been shown to be positively correlated with the population density of Village Bay (Hayward *et al.* 2014) and is also dependent on larvae counts in a sheep's home range (Wilson *et al.* 2004). Lambs have high FEC levels, and males tend to have higher FEC than females (Wilson *et al.* 2004; Craig *et al.* 2008). In adults older than 2 years, FEC increases with age in males while in females FEC decreases initially and rises again in later life (Hayward *et al.* 2009). There is also evidence that strongyle infection has negative impacts on the sheep. Post-mortems on the Soay sheep have also found that the abomasum of sheep were inflamed and lesions were present associated with damage by parasites which is likely to contribute to mortality in malnourished hosts (Gulland 1992). In addition, strongyle FEC is negatively associated with weight (Craig *et al.* 2008), and negatively predicts survival in lambs (Hayward *et al.* 2011).

1.5.4 Immunology

Studies into the causes and consequences of variation in immunity in the Soay sheep on St Kilda are greatly aided by the presence of reagents and tools developed for their domestic counterparts. These studies have documented differences in immune measures between the sexes, and age-related decreases in some T cell subtypes (naïve T cells, gamma-delta T cells), while parasite-specific antibody levels, eosinophils and other T cell subsets (CD8+ and CD4+) increased with age (Nussey *et al.* 2012; Watson *et al.* 2016). There is also evidence that immune responses are important in parasite resistance in this population; high anti-*T. circumcincta* IgA and anti-*T. circumcincta* pan-isotype levels are associated with low FEC in lambs and adults respectively (Coltman *et al.* 2001b; Hayward *et al.* 2014). Positive associations between total IgA and IgM levels with weight in adult females, suggests that some immune responses may be indicative of condition, while the negative association between total IgG levels and weight are indicative of costs of immunity (Nussey *et al.* 2014). There is also evidence of trade-offs and selection on immunity in this population. In adult sheep, anti-nuclear antibody levels were associated with reduced reproductive success in both adult males and females. However, there was also evidence of environment dependent selection; in crash years anti-nuclear antibody levels were associated with survival of females over-winter, suggesting a mechanism for the maintenance of variation in immunity in the wild (Graham *et al.* 2010). In addition, anti-*T. circumcincta* IgG levels positively predicted over-winter survival suggesting a fitness benefit of anti-helminth immunity (Nussey *et al.* 2014; Watson *et al.* 2016). Studies using more than one immune measure have noted that correlations between immune traits are typically low, producing independent associations with health and fitness measures (Nussey *et al.* 2014; Watson *et al.* 2016). However, relatively little is known about the genetic basis of variation in immune traits in this population.

1.5.5 Quantitative genetics

The Soay sheep population on St Kilda offer an excellent opportunity to investigate the genetic basis of traits in the wild. The relatedness of individuals in the study population is known through a population pedigree, which has been constructing using maternities and paternities assigned with 315 unlinked single nucleotide polymorphisms (linkage

disequilibrium $r^2 < 0.05$) with a minor allele frequency > 0.4 using the R library sequoia (Huisman 2017). This has led to a number of quantitative genetic studies in the Soay sheep, including the determination of the heritability of body size traits (Wilson *et al.* 2007; Bérénos *et al.* 2015), horn size (Johnston *et al.* 2011) as well as maternal effects underlying early life traits (Wilson *et al.* 2005). In addition to a population pedigree, genomic information is available from a high-density SNP chip. In total, 5805 sheep have been genotyped at 51,134 SNPs on the Ovine SNP50 BeadChip (Johnston *et al.* 2016). GWAS carried out using this SNP data have identified regions associated with horn morphology (Johnston *et al.* 2011), leg length (Bérénos *et al.* 2015) and recombination rate (Johnston *et al.* 2016). However, no GWAS has been carried out on immunological traits in this population.

Previous studies on the Soay sheep have documented both significant and non-significant heritabilities of strongyle FEC depending on age group and sample size (Coltman *et al.* 2001a; Beraldi *et al.* 2007; Brown *et al.* 2013). Allelic variation in the MHC has been associated with FEC (Paterson, Wilson & Pemberton 1998) and a polymorphism in IFNγ gene was associated with reduced FEC and increased anti-*T. circumcincta* IgA levels (Coltman *et al.* 2001b). However further candidate gene studies and QTL mapping revealed no significant regions associated with strongyle FEC (Beraldi *et al.* 2007; Brown *et al.* 2013). In comparison, the immune measures anti-nuclear antibody levels and anti-*T. circumcincta* pan-isotype antibody levels have been documented to be significantly heritable (Graham *et al.* 2010; Hayward *et al.* 2014), and were generally more heritable than FEC (Brown *et al.* 2013). This suggests that there may be considerable genetic variation underlying immune responses in this population.

1.6 Rationale & aims of this thesis

In this thesis, I aimed to investigate the causes and consequences of variation in immunity under natural conditions, with a focus on determining the genetic basis and maternal effects underlying immunity. To do so, I measured anti-*T. circumcincta* IgA, IgE and IgG levels. These measures were chosen for multiple reasons. Firstly, *T. circumcincta* is a prevalent parasite with negative effects on Soay sheep (Gulland 1992; Hayward *et al.* 2011) and is also

a prevalent parasite of domestic sheep in temperate regions (McNeilly *et al.* 2009). Anti-*T. circumcincta* antibody levels are also cross-reactive with a number of other antigens from nematode species present in the population (*Trichostrongylus vitrinus* and *Trichostrongylus axei*), in addition to a nematode absent in this population (*Haemonchus contortus*) and a nematode of mice (*Heligmosomoides polygyrus*) (Figure 1.5). This suggests that these antibody levels are binding to conserved helminth antigens shared by numerous nematodes and that these antibody levels provide us with a general measure of anti-helminth immune responses in the Soay sheep. Secondly, antibody responses have been implicated to have an important role in the development of protective immunity in mice-helminth models as well as to *T. circumcincta* in domestic ruminants (McNeilly *et al.* 2009; Harris & Gause 2011). Thirdly, these anti-parasite measures have been shown to be associated with parasite resistance, over-winter survival and trade-offs with reproductive success (Coltman *et al.* 2001b; Hayward *et al.* 2014; Nussey *et al.* 2014; Watson *et al.* 2016). Finally, all three isotypes were measured since IgA, IgE and IgG have all been implicated to some degree in protection against helminths (Harris & Gause 2011) and due to the discovery of low correlations between these levels in Soay sheep (Nussey *et al.* 2014; Watson *et al.* 2016).

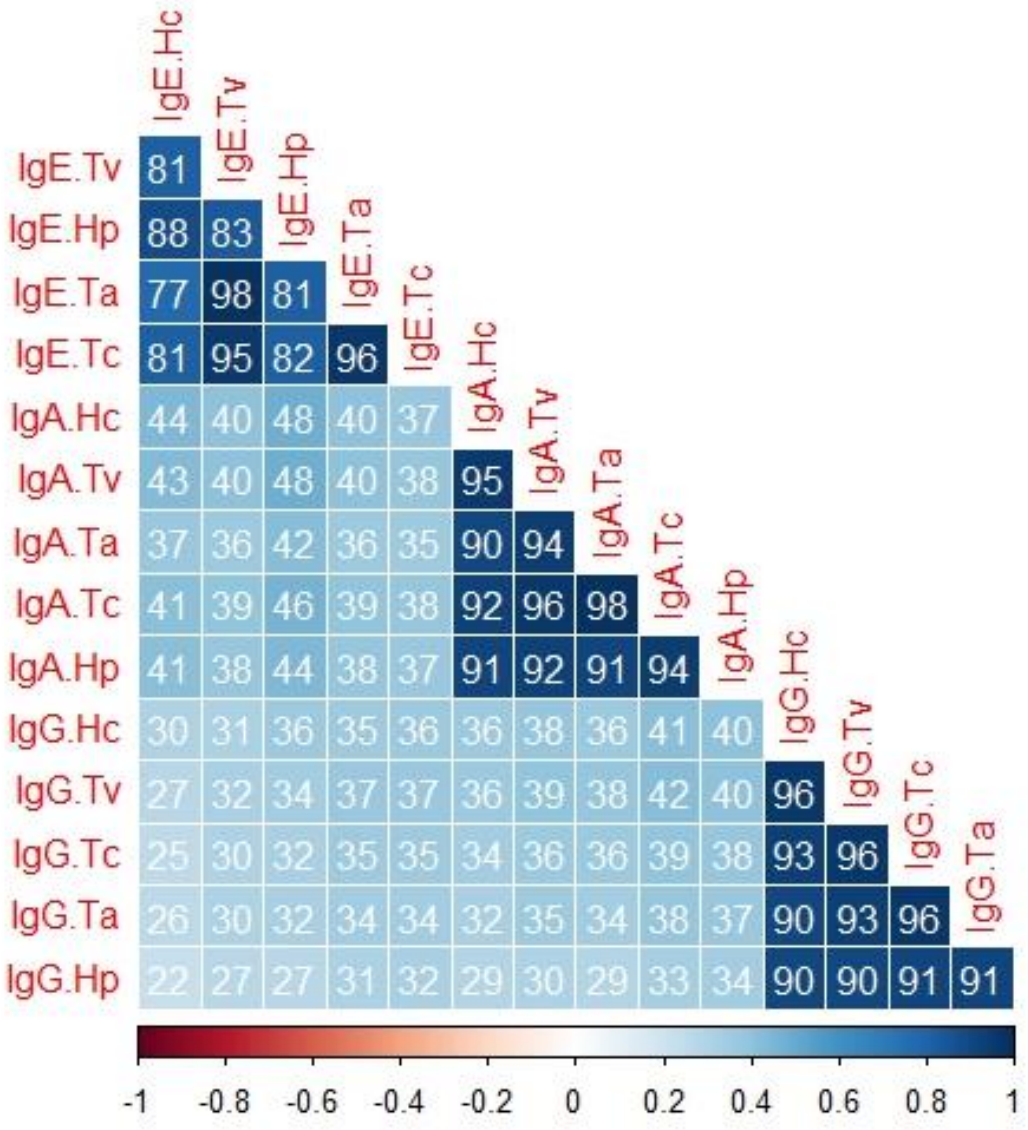

Figure 1.5. Correlation plot showing cross-reactivity of antibodies against L3 anti-*T. circumcincta* (Tc) IgA, IgE and IgG levels with L3 antigens from *Trichostrongylus vitrinus* (Tv), *Trichostrongylus axei* (Ta), *Haemonchus contortus* (Hc), and *Heligmosomoides polygyrus* (Hp) measured in plasma samples from wild Soay sheep. This work was carried out by Kathryn Watt, Dan Nussey, Tom McNeilly & Rick Maizels.

1.7 Thesis overview

In Chapter 2, I carry out a quantitative genetic analysis to determine the heritability and repeatability of anti-helminth antibody levels across a longitudinal dataset from Soay sheep caught over a 25 year period. I determine, using a genome wide association study of ~38,000 SNPs, whether any single nucleotide polymorphisms are associated with anti-helminth antibody levels in lambs and adults, and if present, how much additive genetic and phenotypic variance they explain.

Using the same dataset, in Chapter 3 I perform an analysis to determine the consequences of variation in anti-helminth antibody levels and investigate whether there is evidence for selection acting on these traits or on SNPs associated with antibody levels. Specifically, I test whether anti-helminth antibody levels are associated with body weight, strongyle faecal egg counts, over-winter survival and breeding success. I examine whether age, sex or environment dependent associations between antibodies and health and fitness traits are present.

Chapter 4 is a pilot study into the causes and consequences of variation in maternally transferred anti-helminth antibody levels in neonates and endogenous anti-helminth antibody levels in lambs in a 10-year dataset. I investigate whether offspring and maternal characteristics are associated with variation in antibody levels at these two time points. I use a quantitative genetic approach to quantify the maternal effects, as well as the additive genetic, maternal genetic and maternal environment components explaining variance in antibody levels at these time points. Finally, I test whether anti-helminth antibody levels in early life are associated with neonate survival, first winter survival or weight and strongyle faecal egg counts at four months old.

Following on from the exciting results from the pilot study in Chapter 4, maternally transferred anti-helminth antibody levels in neonates caught close to birth were measured in a much larger set of blood samples collected across 25 years. In Chapter 5 using this larger dataset, I look to confirm maternal and offspring predictors of variation in maternal antibody transfer and generate reliable estimates of the additive genetic, maternal genetic and maternal

environment components underlying variance in maternal antibody levels. Further, using a measure of total protein transferred as well as an additional antibody isotype measure, I test whether any associations between maternal antibody levels and health and fitness traits (weight, strongyle faecal egg counts, neonate survival or winter survival) are due to parasite-specific antibody measures or are likely due to total protein transferred. Lastly, I investigate whether there is any evidence of costs for mothers in maternal antibody transfer, by looking at associations of maternal antibody levels with her weight, strongyle faecal egg counts, winter survival and breeding success.

Chapter 2

The genetic architecture of helminth-specific immune responses in a wild mammal

2.1 Summary

Host-parasite interactions are powerful drivers of evolutionary and ecological dynamics in natural populations. Variation in immune responses to infection is likely to shape the outcome of these interactions, with important consequences for the fitness of both host and parasite. However, little is currently known about how genes contribute to variation in immune responses under natural conditions, despite the importance of this for our understanding of the evolutionary forces shaping individual differences in immunity and the evolution of host-parasite interactions. The wild Soay sheep population on St Kilda have been individually monitored for over 30 years, and are heavily infected with strongyle gastrointestinal nematodes. In this study, we assayed a total of 6,543 plasma samples from 3,190 sheep caught over 25 years for levels of IgA, IgE and IgG antibodies against the prevalent nematode *Teladorsagia circumcincta*. Using the population pedigree and Ovine SNP chip data, we estimated the within-individual repeatability and heritability of immune phenotypes in different age groups, and performed a genome-wide association study (GWAS) to test whether genes or genomic regions of large effect could be detected. All three antibody levels were highly repeatable across an individual's lifetime, with all three isotypes being significantly heritable (IgA: 0.36±0.03, IgE: 0.18±0.02 and IgG: 0.15±0.02). Maternal, birth year and capture year effects explained a small proportion of the variances in all isotypes in both lambs and adults. In addition, while a genome wide association study found no significant associations between single nucleotide polymorphisms and IgE and IgG levels, IgA levels were associated with 12 single nucleotide polymorphisms in a region on chromosome 24. The most significant SNP explained 28% of the additive genetic variance and 10% of the phenotypic variance in IgA levels. This study provides evidence of high

repeatability and heritability of a complex immunological trait under natural conditions and provides the first evidence from a genome-wide study that large effect genes outside the MHC region exist for immune traits in nature.

2.2 Introduction

A growing number of studies in humans have shown that immune phenotypes are heritable, with evidence that genes of relatively large effect may underpin that heritability (Evans, Frazer & Martin 1999; Orrù *et al.* 2013; Ye *et al.* 2014; Brodin *et al.* 2015; Roederer *et al.* 2015). In humans, around 20-40% of immunological variation is determined by genetic variation (Liston *et al.* 2016) and a number of genetic variants have been identified in genome wide association studies (GWAS) (Orrù *et al.* 2013; Roederer *et al.* 2015). Studies in domestic livestock have also shown that immune traits are heritable (Christe *et al.* 2001; Strain *et al.* 2002; Clapperton, Glass & Bishop 2008; Murphy *et al.* 2010; Flori *et al.* 2011; Thompson-Crispi *et al.* 2012; Denholm *et al.* 2017) and associated with certain genomic regions (Edfors-Lilja *et al.* 1998; Davies *et al.* 2006; Lu *et al.* 2013; Thompson-Crispi *et al.* 2014). However, relatively little is currently known about how genetic variation underpins the large variation seen in immune responses under natural conditions. Wild animals are typically exposed to a range of micro- and macroparasites and experience a more variable and challenging environment than livestock and Western humans (Maizels & Nussey 2013). Recent rodent studies show that experiencing a more natural environment radically alters immune phenotypes (Abolins *et al.* 2011, 2017; Beura *et al.* 2016), whilst a more variable and challenging environment could dramatically alter both the heritability of immune phenotypes and the importance of particular genes for resistance to infection. Only by studying wild populations, where natural selection is occurring, is it possible to determine how so much genetic variation in immune responses is maintained under natural selection (Maizels & Nussey 2013). Studies in wild birds investigating the heritability of immune responses have reported small and non-significant (Pitala *et al.* 2007; Morrison, Ardia & Clotfelter 2009; Sakaluk *et al.* 2014) as well as moderate and significant heritabilities (Bonneaud *et al.* 2009; Morrison *et al.* 2009; Drobniak *et al.* 2010; Kim *et al.* 2013). However, these studies measure broad, non-specific immune phenotypes such as the phytohaemagglutinin (PHA) response or haematocrit levels, rather than immune responses to ecologically-relevant parasites (Kennedy & Nager 2006; Owen & Clayton 2007). Estimates

of heritability are particularly rare in wild mammals (Graham *et al.* 2010; Hayward *et al.* 2014), and identification of genetic loci in the wild has been limited to candidate gene studies (Bonneaud *et al.* 2009; Turner *et al.* 2011; Gangoso *et al.* 2011; Brown *et al.* 2013). Here, we estimate the repeatability and heritability of anti-helminth antibody levels in a wild ruminant and perform the first GWAS for an immune trait in a wild population.

While genetic effects drive persistent among-individual differences in immune phenotypes, within-individual variation in immunity associated with recent exposure to parasites, nutritional state, and age are well documented in many systems (Palacios *et al.* 2011; Simon *et al.* 2015). To separate among and within individual contributions to phenotypic variation, longitudinal data across the entire lifetime is required. Immunological studies in humans, livestock and laboratory rodents tend to focus on specific age groups, meaning that little is known about how repeatable immune traits are, and whether the genetic architecture underlying these traits remains consistent, across age groups. A growing number of longitudinal studies in humans has found that the high immunological diversity observed in human populations is maintained by high inter-individual variation but stable immune profiles of individuals across longitudinal sampling (Orrù *et al.* 2013; Tsang *et al.* 2014; Brodin *et al.* 2015; Carr *et al.* 2016). Even after acute infections, this stable equilibrium is maintained, with individuals returning to a steady state after infection clearance (Carr *et al.* 2016). Studies in livestock have also found that cellular based immune traits in cattle are highly repeatable, while antibody-based traits were less so (Banos *et al.* 2013; Denholm *et al.* 2017). Dissecting the relative contributions of among- and within-individual variation in immune phenotype using longitudinal data collected in natural systems is a crucial step towards our understanding of the evolutionary and ecological causes and consequences of observed variation in immunity in the wild.

A key question in quantitative genetic studies of wild populations is to what degree the heritability of phenotypic traits is determined by many genes of small effect or whether some are controlled by a few genes or regions that have a large effect (Slate *et al.* 2010; Kardos *et al.* 2016). Early genomic studies in a limited number of natural populations used sparse genetic markers to identify quantitative trait loci (QTL) (Slate 2005). Today, with the accumulation of affordable genomic resources for non-model species, new single nucleotide polymorphism (SNP) based genome wide association studies (GWAS) in natural populations are beginning to emerge, allowing questions to be asked in a handful of extremely well-

studied natural populations (Slate *et al.* 2010; Ellegren 2014). Whilst identifying loci underlying Mendelian traits has met with some success due to their large effect sizes (Johnston *et al.* 2011; Barson *et al.* 2015), GWAS studies on quantitative traits in the wild typically find no, or few, associations between SNPs and phenotypic traits in the wild (Johnston *et al.* 2014; Bérénos *et al.* 2015; Husby *et al.* 2015; Santure *et al.* 2015; Wenzel *et al.* 2015; Kardos *et al.* 2016; Silva *et al.* 2017). This is because studies in the wild tend to be underpowered as a result of small sample sizes, low linkage disequilibrium between typed SNPs and causal variants, or where the effect sizes of causal variants are low, which is common in polygenic traits with many genes contributing to heritability (Slate *et al.* 2010). Studies which have found associations have identified QTL with relatively large effects (Tarka *et al.* 2010; Bérénos *et al.* 2015; Husby *et al.* 2015; Johnston *et al.* 2016). However, small sample sizes may lead to over-estimation of allele effect sizes (known as the Beavis effect) or may lead to false associations between SNPs and phenotypes due to chance, particularly at rare alleles (Beavis 1994; Slate 2013). While QTL studies to date in animals and plants have suggested that only a few loci with pleiotropic effects are associated with parasite resistance (Wilfert & Schmid-Hempel 2008), only one GWAS study has been performed on parasite burden in the wild (Wenzel *et al.* 2015). In this study, *Trichostrongylus tenuis* parasite burden in red grouse was associated with excess heritability on three chromosomes, suggesting that host resistance is intermediate between a polygenic and oligogenic trait (Wenzel *et al.* 2015). However, parasite burden is not fully indicative of the immune response to parasites, and parasite burden may vary due to exposure, age, sex, social status, environment and condition (Schmid-Hempel 2011). Previous studies investigating the genetic basis of immune responses in the wild have used a candidate gene approach which has implicated a role for variation in major immune genes such as the MHC and cytokine genes on immune measures (Coltman *et al.* 2001b; Bonneaud *et al.* 2009; Turner *et al.* 2011; Brown *et al.* 2013). However, these studies focus on only a small section of the genome, and studies have suggested that non-MHC regions explain a considerable proportion of the variation in immune responses to antigens (Jepson *et al.* 1997; Acevedo-Whitehouse & Cunningham 2006). It is therefore unknown whether immune responses to naturally occurring parasites in the wild are attributable to many small effect genes, or whether regions of large effect are present.

Sheep and their gastrointestinal strongyle nematodes represent a well-understood host-parasite system, due to the agricultural and economic importance of domestic sheep

(Benavides *et al.* 2016). Gastrointestinal helminth infections are considered the largest health threat for grazing ruminants and the estimated annual cost of GI infections in sheep alone is estimated at £84 million, which is exacerbated by the increasing prevalence of anthelmintic resistance (Nieuwhof & Bishop 2005; Jackson & Miller 2006). Following evidence of host resistance to infection, there has been a surge of interest in determining the genes underlying host resistance (Benavides *et al.* 2016). Host resistance to nematodes is typically assessed by measuring faecal egg count (FEC) as an indirect and non-invasive measure of parasite burden following parasite challenge (Dominik 2005). Heritability of strongyle nematode FEC has been found to range between 0.04-0.44 in lambs (Bouix *et al.* 1998; Gauly & Erhardt 2001; Bishop *et al.* 2004; Wolf *et al.* 2008; Riggio *et al.* 2013) and between 0.09-0.25 in adult ewes (Bouix *et al.* 1998; Bishop & Stear 2001; Gutiérrez-Gil *et al.* 2010). Of the strongyle parasites, *Teladorsagia circumcincta* is of major economic importance for domestic sheep in temperate regions (McNeilly *et al.* 2009). In domestic sheep, defence against *T. circumcincta* in lambs is associated with parasite-specific IgA responses directed at worm growth and subsequent female fecundity (Stear *et al.* 1995; Strain *et al.* 2002), while in animals older than 8 months a hypersensitive response results in expulsion of incoming larvae from the mucosa (McNeilly *et al.* 2009). Heritability of anti-*T. circumcincta* IgA levels have been estimated as 0.27-0.30 against L3 (Riggio *et al.* 2013), and 0.56 against L4 stages of the parasite in lambs (Strain *et al.* 2002), while heritabilities appear to be lower in adults at 0.19 (Gutiérrez-Gil *et al.* 2010). Across both candidate gene and genome-wide studies, 126 markers and marker intervals have been significantly associated with FEC or protective immunological traits to the three main GI nematodes of sheep, with candidate gene studies primarily focussed on the interferon gamma (IFNγ) gene on chromosome 3 and the major histocompatibility complex (MHC) region on chromosome 20 (Benavides *et al.* 2016). Due to the focus on identifying individuals for selective breeding purposes and the greater impact of parasite infections in lambs, most studies have focussed on this age group, with only a few studies looking at adult ewes (Bishop & Stear 2001; Gutiérrez-Gil *et al.* 2009, 2010; Atlija *et al.* 2016). This bias could lead to a lack of discovery of sex and age-dependent genetic effects. In support of this, differences in resistance loci between studies have raised the possibility that the genetic control of these mechanisms may differ between adult sheep and lambs (Atlija *et al.* 2016). Since adult females may contribute massively to larval counts during the periparturient rise, and because adult males often have higher parasite burdens than females, it is crucial to understand the genetic basis of resistance in older animals (Zuk & McKean 1996; McRae *et al.* 2015).

The long-term study of wild Soay sheep on St Kilda provides a powerful opportunity to apply genomic and immunological tools developed for the study of domestic sheep under natural conditions (Johnston *et al.* 2013; Hayward *et al.* 2014; Nussey *et al.* 2014; Bérénos *et al.* 2015; Watson *et al.* 2016). In this study, we combine these tools to dissect the genetic architecture of anti-parasite antibody levels measured over the lifetimes of individuals over a twenty-five year study period. Soay sheep on St Kilda are infected with a variety of gastrointestinal strongyle nematodes common to domestic sheep, predominantly *Teladorsagia circumcincta, Trichostrongylus axei* and *Trichostrongylus vitrinus* (Wilson *et al.* 2004; Craig *et al.* 2006). Strongyle nematode burden, in combination with harsh winter weather and low food availability, are a strong selective force on the sheep (Gulland 1992; Wilson *et al.* 2004; Craig *et al.* 2006; Hayward *et al.* 2011). Previous estimates of the heritability of strongyle faecal egg count have been modest, and not consistently significantly different from zero, depending on sample size and age group (Coltman *et al.* 2001a; b; Beraldi *et al.* 2007). Parasite-specific antibody responses are moderately and significantly heritable (Brown *et al.* 2013; Hayward *et al.* 2014) and parasite specific IgA levels and parasite-specific pan-isotype antibody levels have been shown to be negatively associated with strongyle faecal egg count (Coltman *et al.* 2001b; Hayward *et al.* 2014). Previous QTL mapping and candidate gene studies of parasite egg counts and pan-isotype antibody levels have failed to identify significant loci (Beraldi *et al.* 2007; Brown *et al.* 2013), however, a polymorphism at the IFNγ locus in lambs was associated with reduced faecal egg counts and increased parasite-specific IgA levels (Coltman *et al.* 2001b). In two recent studies, we demonstrated that different isotypes of anti-*T. circumcincta* antibodies were weakly correlated with one another and that levels of the IgG isotype were positively associated with subsequent survival (Nussey *et al.* 2014; Watson *et al.* 2016). Here, we measured anti-*T. circumcincta* IgA, IgE and IgG levels from plasma samples collected from Soay sheep over a 25 year period, and combine these data with the population pedigree and OvineSNP50 BeadChip data to: (i) determine the repeatability and heritability of these immune phenotypes and (ii) to conduct a GWAS to identify any particular genes or genomic regions responsible for variation in these immune traits.

2.3 Methods

2.3.1 Study population

The Soay sheep is a primitive breed of domestic sheep that was isolated on the island of Soay in the remote St Kilda archipelago several millennia ago, and has been living in unmanaged conditions since then (Clutton-Brock & Pemberton 2004). In 1932, just over 100 Soay sheep were moved to the larger island of Hirta after the evacuation of all human residents. The population expanded and now fluctuates between approximately 1,000 and 2,000. Approximately a third of the Hirta population lives in the Village Bay area, and these individuals have been the subject of a long-term study since 1985 (Clutton-Brock & Pemberton 2004). In April, around 95% of all lambs born in this area are caught each year and individually tagged. Each August, as many sheep as possible from the study population are re-captured using temporary traps (Clutton-Brock & Pemberton 2004). At capture, animals are weighed and blood and faecal samples are collected. Whole blood samples are collected into heparin tubes, centrifuged at 3000 r.p.m. for 10 minutes, and plasma removed and stored at -20^{o}C. Strongyle faecal egg count (FEC) is estimated from faecal samples as the number of eggs per gram using a modified McMaster technique (Gulland & Fox 1992). Contributing to the majority of the strongyle FEC burden is *Teladorsagia circumcincta*, *Trichostrongylus axei* and *Trichostrongylus vitrinus* (Craig *et al.* 2006).

In this analysis, we included all animals that were caught and blood sampled in August between 1990 and 2015, comprising 6543 samples from 3190 individuals. Five samples from late-born lambs caught in August within 50 days of birth were excluded from the dataset, due to the potential presence of maternal antibodies and differences in development stage to other lambs.

2.3.2 Laboratory methods

IgA, IgG and IgE activity against antigens of the third larval stage of *Teladorsagia circumcincta* was measured using direct (IgA, IgG) and indirect (IgE) ELISAs (henceforth, “anti-Tc antibodies”). We used *T. circumcincta* L3 somatic antigen, provided by the Moredun Research Institute, as the capture antigen for all three assays diluted to 2µg/ml in

0.06M Carbonate buffer at pH 9.6. 50µl of the diluted capture antigen was added to each well of a Nunc-immuno 96-microwell plate, which was covered and incubated at 4°C overnight. After washing the wells three times in Tris-buffered saline-Tween (TBST) using a plate washer, 50µl of the Soay sheep plasma sample diluted to 1:50 for IgA and IgE, and 1:12800 for IgG was added to each well. The plates were then covered and incubated at 37°C for 1 hour. Plates were then washed five times with TBST and 50µl per well of rabbit polyclonal anti-sheep IgA detection antibody conjugated to horseradish peroxidase (HRP) (AbD Serotec AHP949P) diluted 1:16000 was added to the anti-*T. circumcincta* IgA assay and 50µl per well of rabbit polyclonal anti-sheep IgG detection antibody conjugated to HRP (AbD Serotec 5184-2104) diluted 1:16000 was added to the anti-*T. circumcincta* IgG assay. For the anti-*T. circumcincta* IgE assay, 50µl per well of anti-sheep IgE (mouse monoclonal IgG1, clone 2F1, provided by the Moredun Research Institute) diluted 1:100 was added, followed by 1 hour incubation at 37°C, five washes with TBST and then 50µl per well of goat polyclonal anti-mouse IgG1-HRP detection antibody (AbD Serotec STAR132P) was added diluted to 1:8000 in TBST. All plates were then incubated at 37°C for 1 hour. Plates were then washed five times with TBST and 100µl of SureBlue TMB 1-Component microwell peroxidase substrate (KPL) was added per well and left to incubate for 5 minutes in the dark at 37°C. Reactions were stopped by adding 100µl per well of 1M hydrochloric acid and optical densities (OD) were read immediately at 450nm using a Thermo Scientific GO Spectrophotometer.

All results were recorded as OD values. To minimise confounders of capture year and age effects with plate to plate variation, each plate included samples from two years paired at random with different age groups on each plate. All plates were run in duplicate and duplicate sample ODs were removed if the coefficient of variation was > 0.2 and the difference between ODs was greater than 0.2. We also checked the correlation of ODs across duplicate plates and re-ran both plates if $r < 0.8$. We included two sample free wells (50µl TBST) as blanks and two wells of positive controls on each plate. Positive controls for the IgE assay were serum from ewes trickle-infected with *T. circumcincta* and for the IgA and IgG assay were plasma from normal healthy non-immunised domestic sheep. For subsequent analyses, the mean optical density ratio of each sample was taken according to this formula:

$$OD = \frac{(\text{sample OD} - \text{blank OD})}{(\text{positive control OD} - \text{blank OD})}$$

Where the numerator was set to zero if the blank OD was greater than the sample OD in order to avoid negative values. Distributions of antibodies are shown in Figure 2.1. The number of samples that failed quality control per assay was 13 for IgA (7 lambs and 6 adults), 8 for IgE (6 lambs and 2 adults) and 27 for IgG (5 lambs and 22 adults).

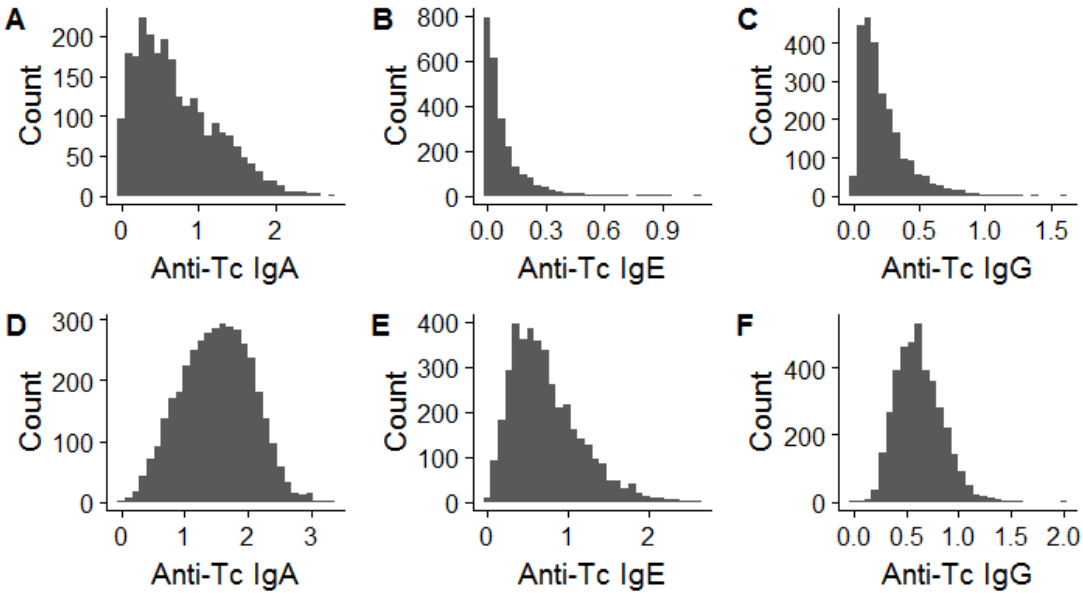

Figure 2.1. Histograms of anti-*Teladorsagia circumcincta* IgA, IgE and IgG levels in lamb (A-C) and adult (D-F) Soay sheep.

2.3.3 Statistical analyses

Animal model

We fitted quantitative genetic "animal models" in order to determine the heritability and repeatability of anti-Tc antibody levels in ASReml-R 3.0 (Butler *et al.* 2009). The pedigree used in the animal model was constructed using maternities and paternities assigned with 315 unlinked single nucleotide polymorphisms (linkage disequilibrium $r^2 < 0.05$) with a minor allele frequency > 0.4 using the R library *sequoia* (Huisman 2017). This pedigree included all cohorts from 1985-2015 and includes 8221 individuals with 7142 maternities and 5456 paternities.

Univariate animal models were fitted for each of the three antibody measures for three different age classes: all ages, lambs only and adults (where adults are sheep ≥ 12 months old). Separation of models with and without lambs was due to a large difference observed in antibody levels between lambs and yearlings (see Figure 2.1 & Results) and due to the expected immaturity of the immune response in 4-month-old lambs. The fixed effect structure for the lamb-only model included sex and age in days, while the random effects included the additive genetic component, maternal identity, birth year, ELISA plate number and ELISA run date. The fixed effect structure for the all ages and adult models included sex and age in years (fitted as a factor), while the random effects included capture year and permanent environment (i.e. individual identity, or repeated measures) in addition to the random effects included in the lamb model. Significance of random effects was determined by dropping each random effect from a model containing all random effects and performing a likelihood ratio test on the two log likelihoods.

The proportion of the phenotypic variance explained by each random effect was estimated as the ratio of the relevant variance component to total phenotypic variance. The heritability of each measure was determined as the ratio of the additive genetic variance to the total phenotypic variance. The repeatability of each measure in the adult and all age models was determined as the ratio of the sum of the additive genetic and permanent environment variance to the total phenotypic variance. Standard errors of ratio components were calculated using the pin function from the R package "nadiv" (Wolak 2012).

Genome-wide association study

A genome-wide association study was used to determine if there were associations between individual single nucleotide polymorphisms (SNPs) and the three antibody levels using the package GenABEL v1.8-0 (Aulchenko *et al.* 2007) in R v3.3.3. A total of 6,825 sheep have been genotyped at 51,135 SNPs on the Ovine SNP50 BeadChip. Quality control was carried out using the check.marker function in GenABEL using the following thresholds: SNP minor allele frequency (MAF) > 0.01, SNP locus genotyping success > 0.95, individual sheep genotyping success > 0.95, identity by state with another individual ≥ 0.95. Heterozygous genotypes at non-pseudoautosomal X-linked SNPs within males were scored as missing. Following quality control, 37,664 SNPs from 6,728 sheep remained. All SNP locations were taken from their estimated positions on the sheep genome assembly Oar_v3.1 (GenBank assembly ID GCA_000298735.1)(Jiang *et al.* 2014).

Since GenABEL is unable to model repeated measures, analyses were performed on three subsets of the data: lambs (n = 2033-2035), and the first and last measure of the adults (n = 1343-1349). In the lamb model, sex and age in days were included as fixed effects, while in the adult models, sex and age in years as a factor were included as fixed effects. To account for population structure due to relatedness amongst individuals, a kinship matrix was created using GenABEL based on genomic relatedness at autosomes weighted by allele frequency. The GWAS was then run using GenABEL's 'egscore' function including the kinship matrix as a random effect. P-values were corrected for any additional unaccounted population structure by dividing them by the genomic control parameter λ, in cases where λ > 1, to reduce the incidence of false positives. The significance threshold after multiple testing was determined using a linkage disequilibrium based approach with a sliding window of 50 SNPs (outlined in Moskvina & Schmidt, 2008); for a false discovery rate of α = 0.05, the threshold p was set at 2.245×10^{-6}.

SNP effect sizes

To get unbiased estimates of SNP effect sizes, all SNPs meeting the genome-wide significance threshold after correcting for inflation were added individually as covariates with three levels (for the three SNP genotypes) in ASReml-R. Effect sizes were estimated across all ages, with the same model structure used previously (see Animal model). The variance explained by each SNP was calculated using the following equation (Falconer & Mackay 1996):

$$V_{SNP} = 2pq[a + d(q - p)]^2$$

Where p and q are the frequencies of alleles A and B at each individual SNP locus, a is half the difference in the effect size estimated for the genotypes AA and BB, and d is the difference between a and the effect size estimated for genotype AB when fitted as a fixed effect in an animal model. Estimates of standard errors for V_{SNP} were calculated using the delta method. The proportion of additive genetic variance explained by each SNP was calculated as the ratio of V_{SNP} to V_A, where V_A was estimated in a model without the SNP as a covariate. The proportion of phenotypic variance explained by each SNP was calculated as the ratio of V_{SNP} to V_P, where V_P was estimated in a model without the SNP as a covariate. Significance of SNPs within the animal model was tested with conditional Wald tests.

Candidate genes

Where SNPs were significantly associated with antibody levels, candidate genes in the region were located using Ensembl and location of SNPs to genes using Ensembl's BioMart tool based on the sheep genome assembly Oar_v3.1 (Jiang *et al.* 2014). Linkage disequilibrium between SNPs in the region (using r^2) was determined using the R library "LDheatmap" (Shin *et al.* 2006) and linkage disequilibrium between the most significant SNP and other SNPs in the region was determined using GenABEL's 'r2fast' function (Aulchenko *et al.* 2007).

2.4 Results

2.4.1 Phenotype data

August *T. circumcincta*-specific antibody levels of the three isotypes tested (IgA, IgG and IgE) were weakly positively correlated with each other, with slightly stronger correlations in lambs (Figure 2.2). There was a strong association between antibody levels and age, with a pronounced jump in antibody levels between four-month-old lambs, and 16-month-old yearlings (Figure 2.3). In addition, linear mixed models found that males had lower IgA and IgG levels as lambs (Wald tests: IgA: $\chi^2_{(1)} = 9.580$, $p = 0.002$; IgG: $\chi^2_{(1)} = 12.221$, $p < 0.001$), and lower IgG levels as adults (Wald tests: $\chi^2_{(1)} = 43.803$, $p < 0.001$) compared to females (Figure 2.4).

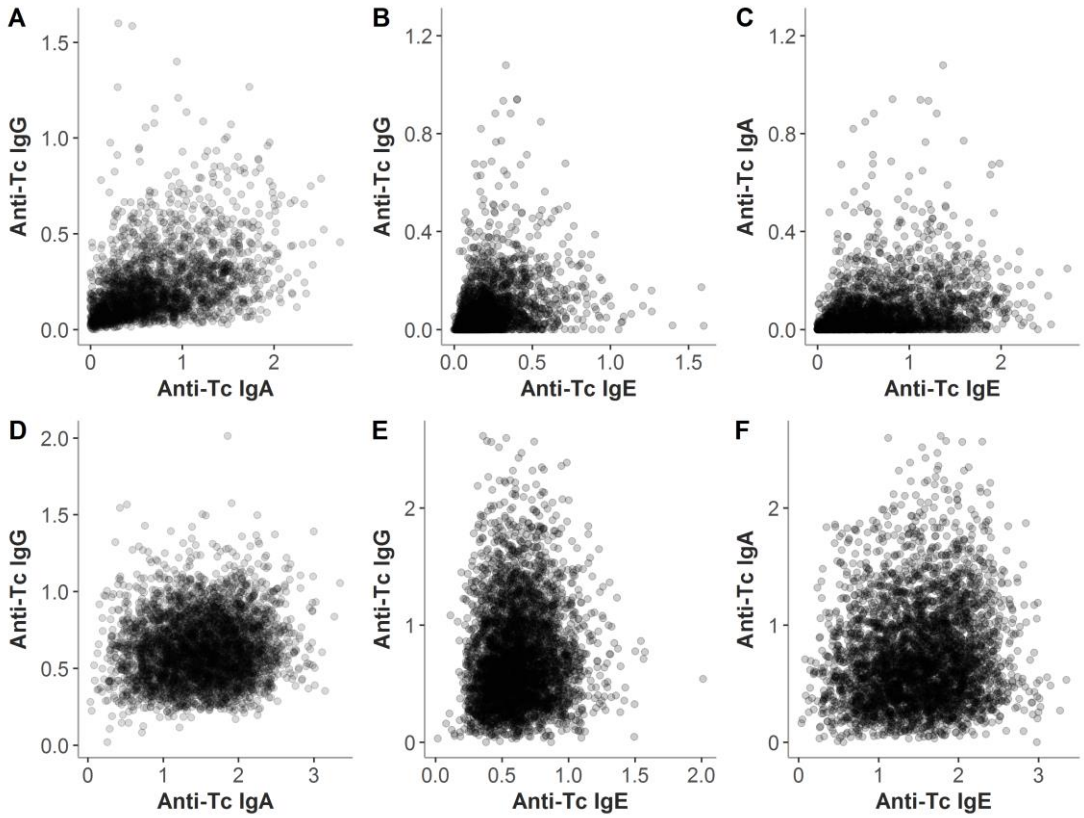

Figure 2.2. Scatterplots of raw data showing correlations between August anti-*T. circumcincta* IgG, IgA, and IgE antibody levels in Soay sheep lambs (A-C) and adults (D-F) (Lambs - IgA & IgG: r = 0.417, $t_{2443} = 22.700$, $p < 0.001$; IgE & IgG: r = 0.269, $t_{2444} = 13.804$, $p < 0.001$; IgE & IgA: r = 0.278, $t_{2442} = 14.285$, $p < 0.001$; Adults - IgA & IgG: r = 0.103, $t_{3913} = 6.468$, $p < 0.001$; IgE & IgG: r = 0.072, $t_{3916} = 4.517$, $p < 0.001$; IgE & IgA: r = 0.113, $t_{3933} = 7.120$, $p < 0.001$).

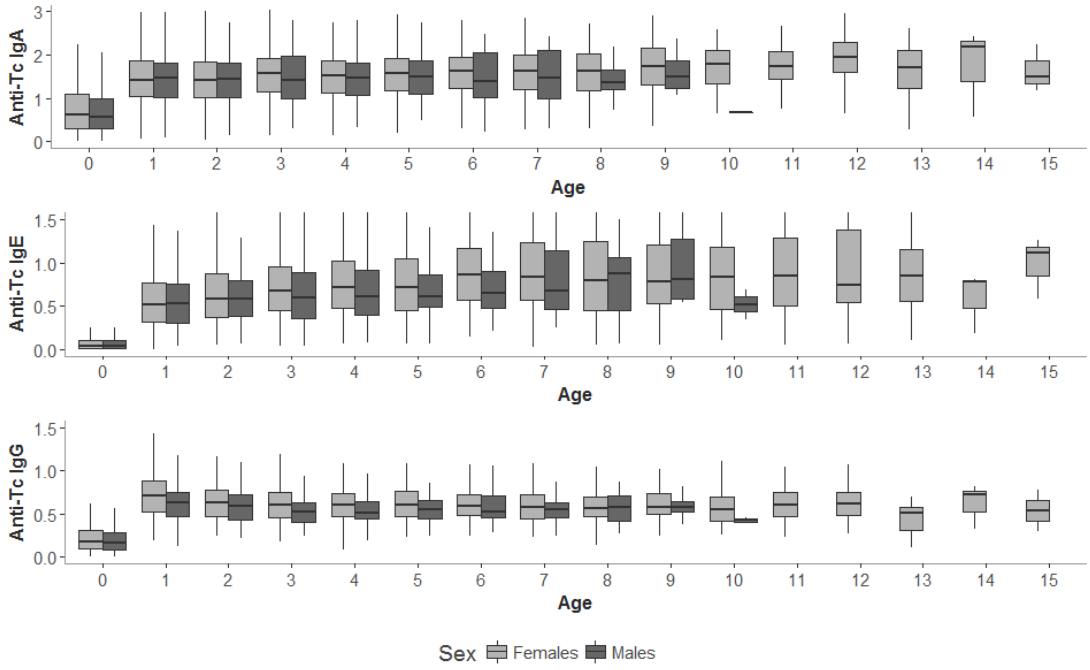

Figure 2.3. Boxplots of raw data showing associations between August antibody levels across all ages for anti-*T. circumcincta* IgA, IgE, and IgG in wild Soay sheep. There were no samples from males aged ≥11 years due to the shorter lifespan of males. Boxes show the median and the interquartile range (IQR) with whiskers extending from the hinges to values no further than 1.5*IQR from the hinge (outliers not shown).

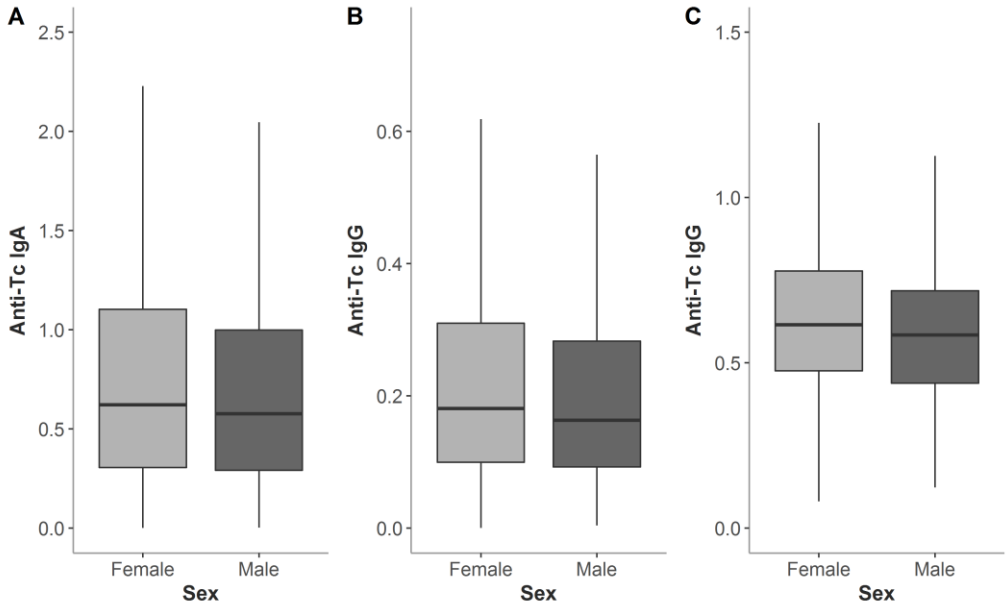

Figure 2.4. Boxplots of raw data showing associations between August antibody levels and sex for anti-*T. circumcincta* IgA and IgG in lambs (A-B) and anti-*T. circumcincta* IgG in adults (C) in wild Soay sheep. Boxes show the median and the interquartile range (IQR) with whiskers extending from the hinges to values no further than 1.5*IQR from the hinge (outliers not shown).

2.4.2 Animal model

All three anti-Tc antibody isotypes were highly repeatable within individuals, with a substantial component of this among individual variation attributable to additive genetic effects (Table 2.1-2.3, Figure 2.5). In models including all sheep, repeatabilities were 0.59±0.02 for IgA, 0.48±0.01 for IgE and 0.35±0.02 for IgG. However, repeatabilities were much higher in adults following exclusion of lamb measures (0.76±0.01 for IgA, 0.74±0.01 for IgE and 0.53±0.02 for IgG). The high stability of antibody measures in adulthood is illustrated by the strong positive correlation between antibody measures taken in two consecutive years (Figure 2.6; IgA: $r = 0.810$, $t_{1930} = 60.684$, $p < 0.001$; IgE: $r = 0.831$, $t_{1936} = 65.704$, $p < 0.001$; IgG: $r = 0.541$, $t_{1913} = 28.159$, $p < 0.001$).

Across all ages, heritabilities were 0.36±0.03 for IgA, 0.18±0.02 for IgE, and 0.15±0.02 for IgG. Variance attributable to capture year and birth year was low (< 6% of the total variance in all models) and maternal effects were weak (< 2% of variance in all models, see Table 2.1- 2.3). In lamb-only models, heritabilities were 0.45±0.05 for IgA, 0.15±0.04 for IgE and 0.26±0.04 for IgG. (Table 2.1- 2.3, Figure 2.5). Year of birth and maternal effects were weak, explaining < 10% of variance in all models. In adults, heritabilities were 0.58±0.04 for IgA, 0.49±0.04 for IgE and 0.24±0.04 for IgG (Table 2.1- 2.3, Figure 2.5). Maternal effects were not significant while year of capture and year of birth effects were again weak (< 2% of variance explained).

Table 2.1. Variance component estimates and their associated ratios for models containing all ages, lambs only, and adults only for levels of anti-*T.circumcincta* IgA measured in St. Kilda Soay sheep. Variances reported are the additive genetic variance (V_A), permanent environment variance (V_{PE}), maternal variance (V_M), birth year variance (V_{BYR}), capture year variance (V_{CYR}), ELISA plate variance (V_{Plate}), ELISA run date variance (V_{Date}) and residual variance (V_R). Included are the variance component estimates ('Est') and the proportion of the total phenotypic variance explained by the term ('Prop') with their associated standard errors in brackets. The significance of random effects was determined by dropping each random effect from a model containing all random effects and performing a likelihood ratio test on the two log likelihoods ('LRT' and 'P'). [B] indicates where variance components have gone to the boundary. Significant effects are shown in bold.

	All					Lambs					Adults				
	Est	**Prop**	**LRT**	**df**	**P-value**	**Est**	**Prop**	**LRT**	**df**	**P-value**	**Est**	**Prop**	**LRT**	**df**	**P-value**
IgA	n=6262	ID=3027				n=2065					n=3832	ID=1337			
V_A	0.100 (0.010)	0.361 (0.030)	**311.457**	**1**	**<0.001**	0.115 (0.014)	0.454 (0.048)	**172.024**	**1**	**<0.001**	0.175 (0.018)	0.577 (0.042)	**237.046**	**1**	**<0.001**
V_{PE}	0.063 (0.007)	0.228 (0.026)	**101.649**	**1**	**<0.001**	-	-	-	-	-	0.054 (0.010)	0.179 (0.036)	**28.968**	**1**	**<0.001**
V_M	0.005 (0.003)	0.018 (0.011)	2.809	1	0.094	0.011 (0.005)	0.045 (0.020)	**6.494**	**1**	**0.011**	<0.001 (<0.001)[B]	<0.001 (<0.001)[B]	N/A	N/A	N/A
V_{BYR}	0.004 (0.002)	0.014 (0.007)	**10.690**	**1**	**0.001**	0.014 (0.005)	0.053 (0.020)	**22.090**	**1**	**<0.001**	0.003 (0.002)	0.011 (0.007)	**5.138**	**1**	**0.023**
V_{CYR}	0.005 (0.002)	0.019 (0.008)	**28.966**	**1**	**<0.001**	-	-	-	-	-	0.002 (0.001)	0.006 (0.004)	**9.215**	**1**	**0.002**
V_{Plate}	0.006 (0.002)	0.022 (0.007)	**61.993**	**1**	**<0.001**	0.005 (0.003)	0.019 (0.011)	**5.261**	**1**	**0.022**	0.007 (0.002)	0.024 (0.008)	**62.816**	**1**	**<0.001**
V_{Date}	0.001 (0.002)	0.002 (0.006)	0.136	1	0.712	<0.001 (<0.001)[B]	<0.001 (<0.001)[B]	N/A	N/A	N/A	0.001 (0.002)	0.003 (0.007)	0.201	1	0.654
V_R	0.093 (0.002)	0.336 (0.012)	-	-	-	0.109 (0.010)	0.429 (0.046)	-	-	-	0.061 (0.002)	0.200 (0.010)	-	-	-

Table 2.2. Variance component estimates and their associated ratios for models containing all ages, lambs only, and adults only for levels of anti-*T.circumcincta* IgE measured in St. Kilda Soay sheep. Variances reported are the additive genetic variance (V_A), permanent environment variance (V_{PE}), maternal variance (V_M), birth year variance (V_{BYR}), capture year variance (V_{CYR}), ELISA plate variance (V_{Plate}), ELISA run date variance (V_{Date}) and residual variance ($V_{\mathbf{R}}$). Included are the variance component estimates ('Est') and the proportion of the total phenotypic variance explained by the term ('Prop') with their associated standard errors in brackets. The significance of random effects was determined by dropping each random effect from a model containing all random effects and performing a likelihood ratio test on the two log likelihoods ('LRT' and 'P'). ^{B} indicates where variance components have gone to the boundary. Significant effects are shown in bold.

	All					Lambs					Adults				
	Est	**Prop**	**LRT**	**df**	**P-value**	**Est**	**Prop**	**LRT**	**df**	**P-value**	**Est**	**Prop**	**LRT**	**df**	**P-value**
IgE	n=6266	ID=3024				n=2064					n=3835	ID=1337			
V_A	0.017 (0.002)	0.180 (0.022)	**151.470**	**1**	**<0.001**	0.003 (0.001)	0.146 (0.035)	**34.744**	**1**	**<0.001**	0.083 (0.010)	0.485 (0.043)	**170.937**	**1**	**<0.001**
V_{PE}	0.028 (0.002)	0.301 (0.021)	**255.197**	**1**	**<0.001**	-	-	-	-	-	0.044 (0.006)	0.256 (0.038)	**47.417**	**1**	**<0.001**
V_M	<0.001 (<0.001)^{B}	<0.001 (<0.001)^{B}	N/A	N/A	N/A	<0.001 (<0.001)^{B}	<0.001 (<0.001)^{B}	N/A	N/A	N/A	<0.001 (<0.001)^{B}	<0.001 (<0.001)^{B}	N/A	N/A	N/A
V_{BYR}	0.001 (0.001)	0.011 (0.006)	**5.260**	**1**	**0.022**	<0.001 (<0.001)	0.016 (0.011)	3.502	1	0.061	<0.001 (<0.001)^{B}	<0.001 (<0.001)^{B}	N/A	N/A	N/A
V_{CYR}	0.001 (0.001)	0.015 (0.006)	**41.215**	**1**	**<0.001**	-	-	-	-	-	0.003 (0.001)	0.015 (0.006)	**43.315**	**1**	**<0.001**
V_{Plate}	0.001 (<0.001)	0.011 (0.004)	**19.966**	**1**	**<0.001**	<0.001 (<0.001)	0.019 (0.011)	**6.540**	**1**	**0.011**	0.002 (0.001)	0.009 (0.003)	**18.656**	**1**	**<0.001**
V_{Date}	<0.001 (<0.001)^{B}	<0.001 (<0.001)^{B}	N/A	N/A	N/A	<0.001 (<0.001)^{B}	<0.001 (<0.001)^{B}	N/A	N/A	N/A	<0.001 (<0.001)^{B}	<0.001 (<0.001)^{B}	N/A	N/A	N/A
V_R	0.045 (0.001)	0.481 (0.013)	-	-	-	0.015 (0.001)	0.819 (0.036)	-	-	-	0.040 (0.001)	0.235 (0.011)	-	-	-

Table 2.3. Variance component estimates and their associated ratios for models containing all ages, lambs only, and adults only for levels of anti-*T.circumcincta* IgG measured in St. Kilda Soay sheep. Variances reported are the additive genetic variance (V_A), permanent environment variance (V_{PE}), maternal variance (V_M), birth year variance (V_{BYR}), capture year variance (V_{CYR}), ELISA plate variance (V_{Plate}), ELISA run date variance (V_{Date}) and residual variance (V_R). Included are the variance component estimates ('Est') and the proportion of the total phenotypic variance explained by the term ('Prop') with their associated standard errors in brackets. The significance of random effects was determined by dropping each random effect from a model containing all random effects and performing a likelihood ratio test on the two log likelihoods ('LRT' and 'P'). [B] indicates where variance components have gone to the boundary. Significant effects are shown in bold.

	All					Lambs					Adults				
	Est	**Prop**	**LRT**	**df**	**P-value**	**Est**	**Prop**	**LRT**	**df**	**P-value**	**Est**	**Prop**	**LRT**	**df**	**P-value**
IgG	n=6247	ID=3027				n=2066					n=3815	ID=1335			
V_A	0.006 (0.001)	0.149 (0.021)	**115.154**	**1**	**<0.001**	0.009 (0.002)	0.256 (0.041)	**82.236**	**1**	**<0.001**	0.011 (0.002)	0.237 (0.038)	**62.502**	**1**	**<0.001**
V_{PE}	0.008 (0.001)	0.202 (0.022)	**111.730**	**1**	**<0.001**	-	-	-	-	-	0.013 (0.002)	0.291 (0.035)	**72.159**	**1**	**<0.001**
V_M	0.001 (<0.001)	0.013 (0.010)	1.587	1	0.208	<0.001 (0.001)	0.014 (0.019)	0.507	1	0.476	<0.001 (<0.001)[B]	<0.001 (<0.001)[B]	N/A	N/A	N/A
V_{BYR}	0.001 (<0.001)	0.026 (0.010)	**26.689**	**1**	**<0.001**	0.002 (0.001)	0.062 (0.025)	**15.870**	**1**	**<0.001**	<0.001 (<0.001)	0.007 (0.006)	1.914	1	0.167
V_{CYR}	0.001 (0.001)	0.031 (0.012)	**50.573**	**1**	**<0.001**	-	-	-	-	-	<0.001 (<0.001)	0.010 (0.007)	**9.881**	**1**	**0.002**
V_{Plate}	0.002 (0.001)	0.052 (0.014)	**141.273**	**1**	**<0.001**	0.002 (0.001)	0.045 (0.017)	**15.899**	**1**	**<0.001**	0.003 (0.001)	0.065 (0.018)	**130.883**	**1**	**<0.001**
V_{Date}	0.002 (0.001)	0.048 (0.021)	**7.286**	**1**	**0.007**	<0.001 (<0.001)[B]	<0.001 (<0.001)[B]	N/A	N/A	N/A	0.004 (0.001)	0.078 (0.029)	**9.055**	**1**	**0.003**
V_R	0.020 (<0.001)	0.480 (0.018)	-	-	-	0.022 (0.001)	0.624 (0.043)	-	-	-	0.015 (<0.001)	0.313 (0.015)	-	-	-

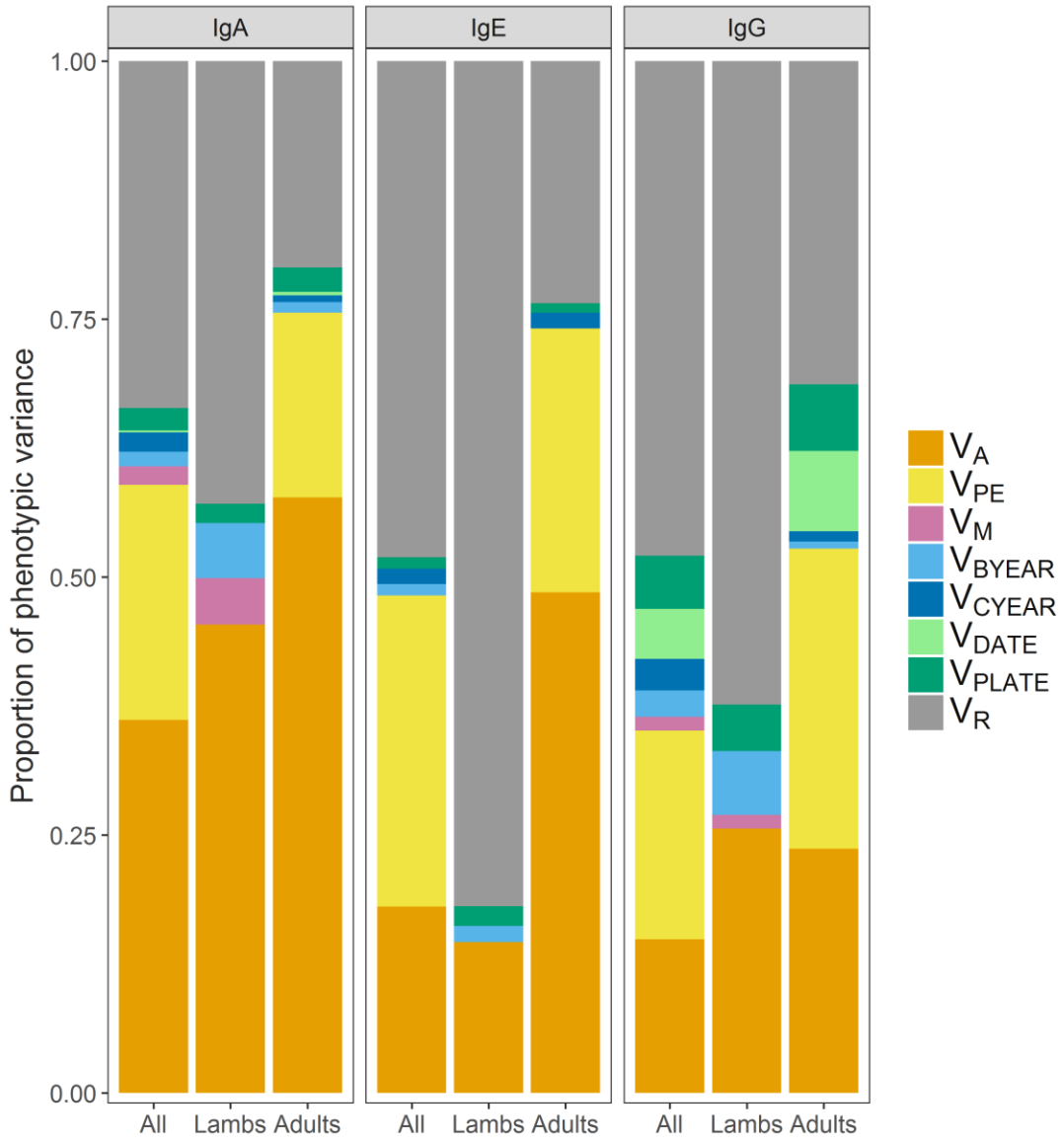

Figure 2.5. The proportion of phenotypic variance explained by different random effects in univariate animal models for the three parasite specific antibody measures in 3 age classes (all ages, lambs and adults > 1 year) in wild Soay sheep. Random effects include the following variance components: additive genetic (V_A), permanent environment (V_{PE}), maternal ID (V_M), birth year (V_{BYEAR}), capture year (V_{CYEAR}), run date of the assay (V_{DATE}), plate number of the assay (V_{PLATE}) and the residual error (V_R).

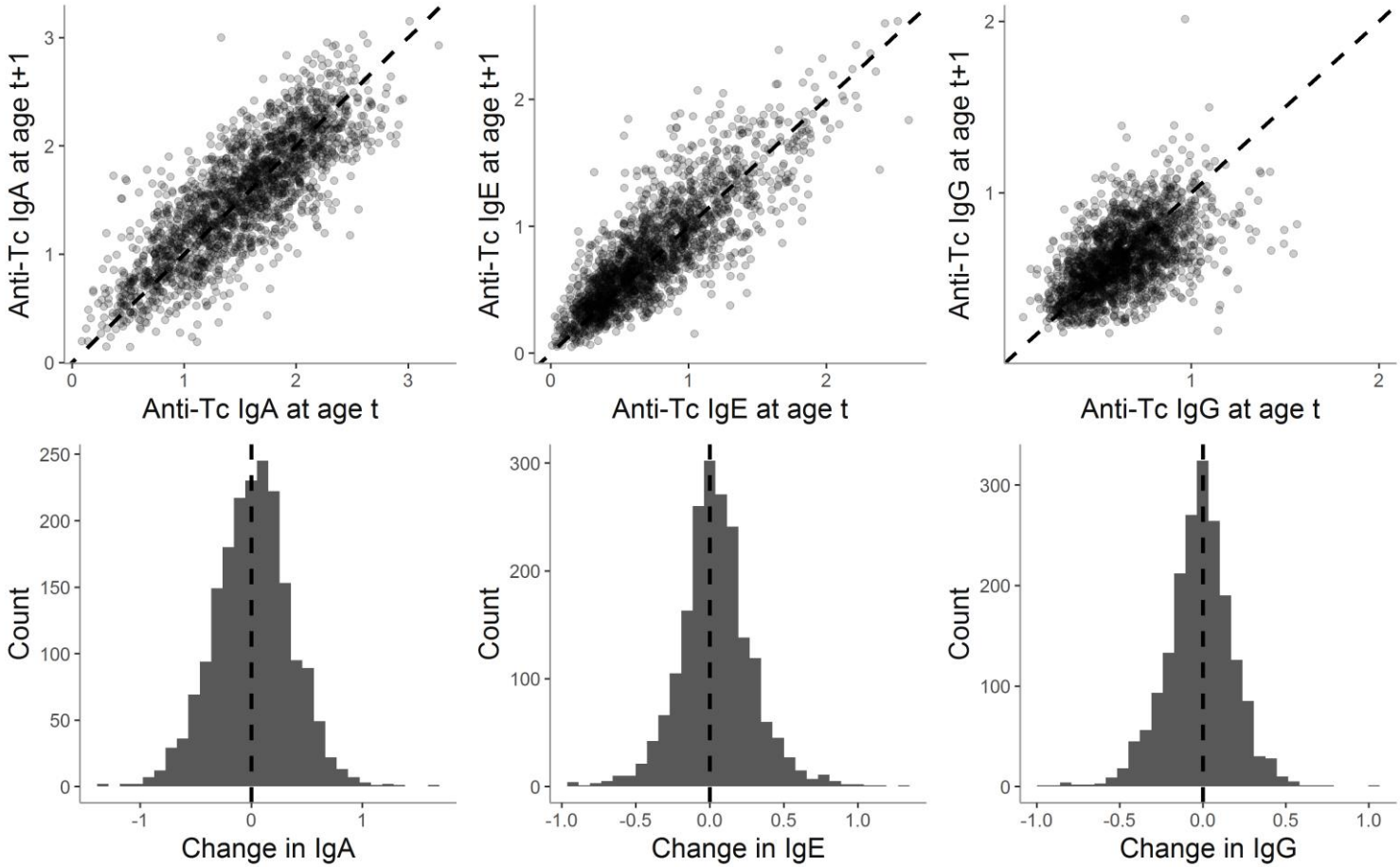

Figure 2.6. Strong temporal correlation in anti-*Teladorsagia circumcincta* IgA, IgE and IgG levels in wild Soay sheep. Scatterplots of all raw data in adults for which there are two antibody measures in two consecutive years with a dashed line indicating a perfect 1:1 relationship. Histograms show the frequency of the change in antibody levels for adults in consecutive years with a dashed line indicating no change.

2.4.3 Genome-wide association study

There was a strong association between anti-*T. circumcincta* IgA levels and a region between ~7.2 -12.1 Mb on chromosome 24 (Figure 2.7, Table 2.4). This was the case for both age groups, with 6 SNPs significant in lambs, 12 SNPs for the first and 9 SNPs for the last adult measure (Figure 2.7, Table 2.4). An additional unmapped SNP was significant at all ages, and was originally mapped to 13.2Mb on chromosome 24 in version 2.0 of the *Ovis aries* genome assembly. The most significant SNP for each age group was OAR24_12006191.1 at 10.5 Mb (Lambs: $p = 8.88x10^{-19}$; First adult: $2.59x10^{-25}$; Last adult: $1.11x10^{-46}$) and the top five SNPs were consistent in all age groups (Table 2.4). No SNPs were significantly associated with anti-*T. circumcincta* IgG or IgE levels (Figure 2.8, 2.9).

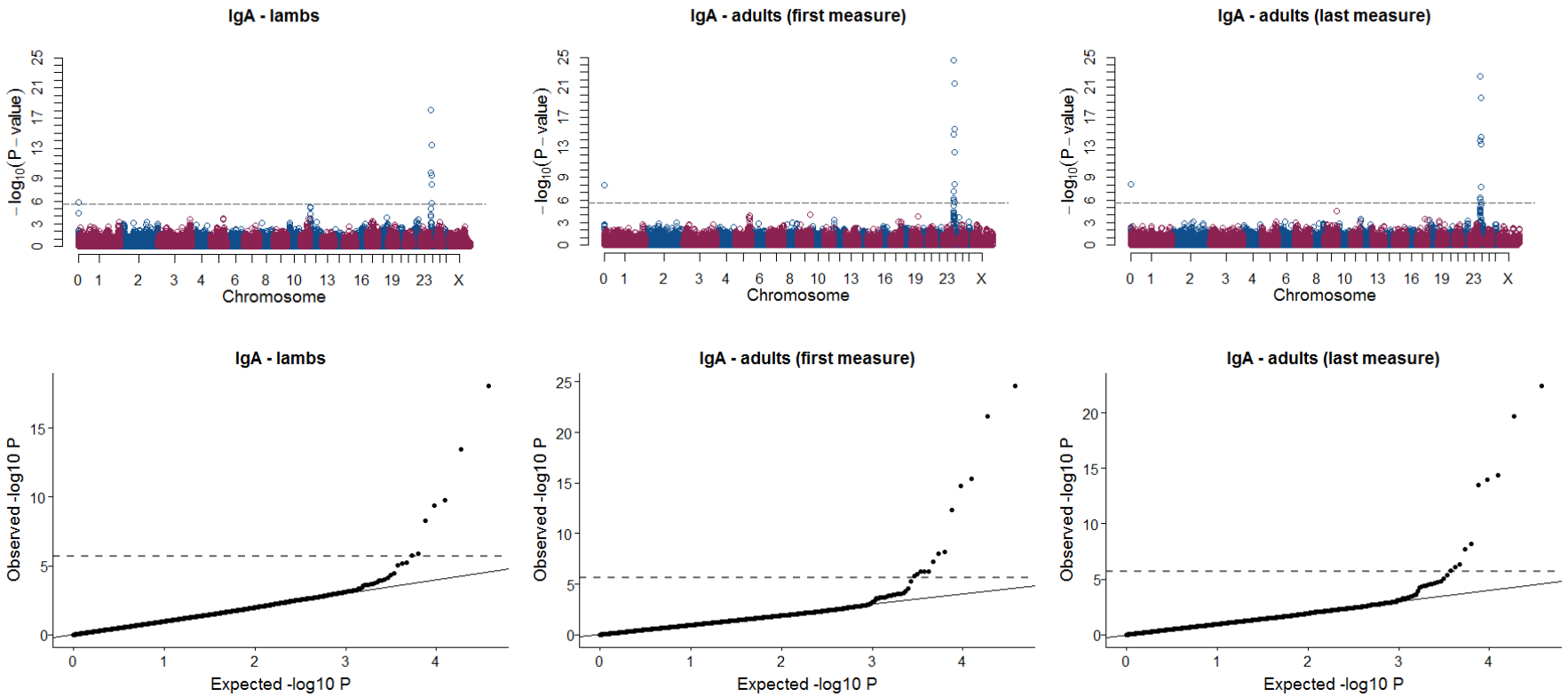

Figure 2.7. Genome-wide association results for anti-*T.circumcincta* IgA levels in Soay sheep in three different age classes (lambs, first adult measure, last adult measure). Manhattan plots of p-values are corrected for inflation (lambda) and the dashed line indicates the Bonferroni genome-wide significance threshold. Q-Q plots showing the association between expected and observed (lambda corrected) P-values. The solid line indicates the one to one line and the dashed line indicates the Bonferroni threshold. Inflation factors were > 1 in all cases.

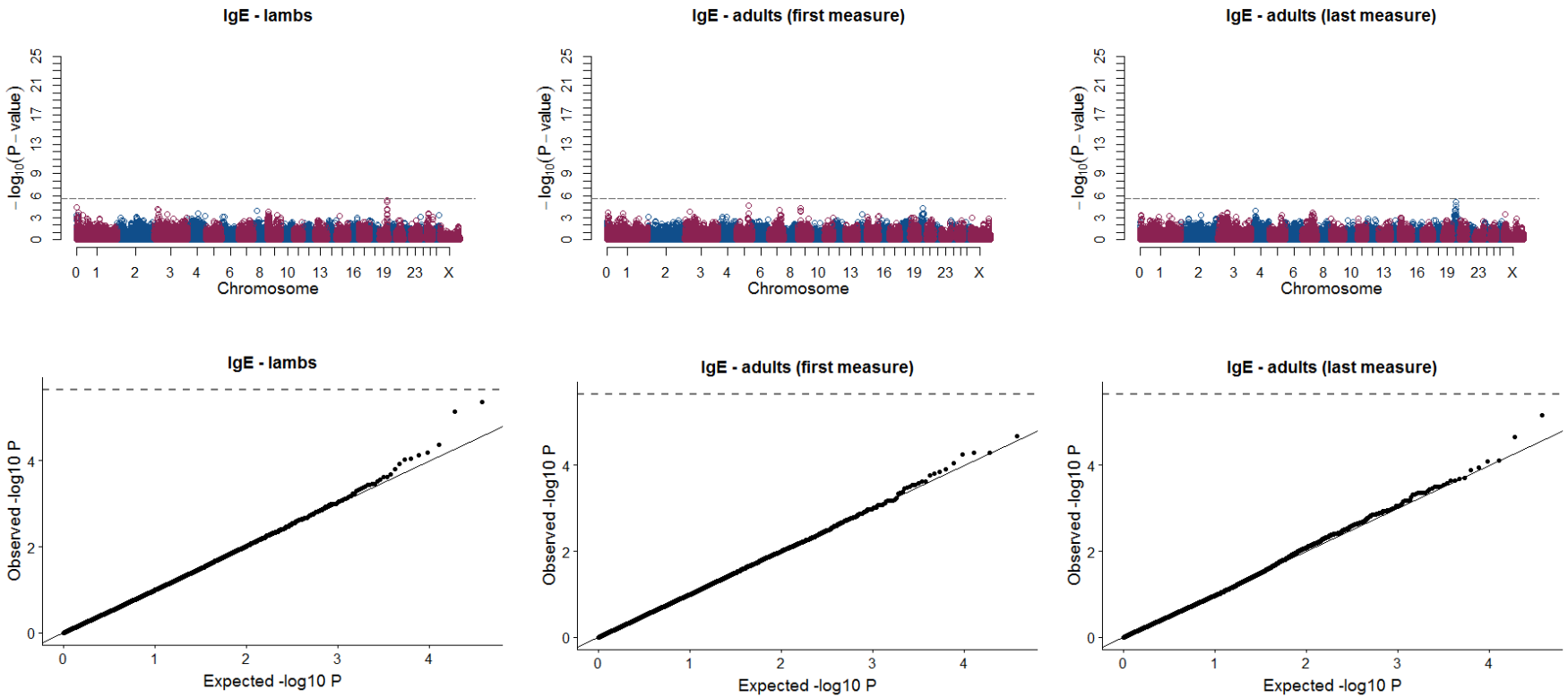

Figure 2.8. Genome-wide association results for anti-*T.circumcincta* IgE levels in Soay sheep in three different age classes (lambs, first adult measure, last adult measure). Manhattan plots of p-values are corrected for inflation (lambda) and the dashed line indicates the Bonferroni genome-wide significance threshold. Q-Q plots showing the association between expected and observed (lambda corrected) P-values. The solid line indicates the one to one line and the dashed line indicates the Bonferroni threshold. Inflation factors were > 1 in all cases.

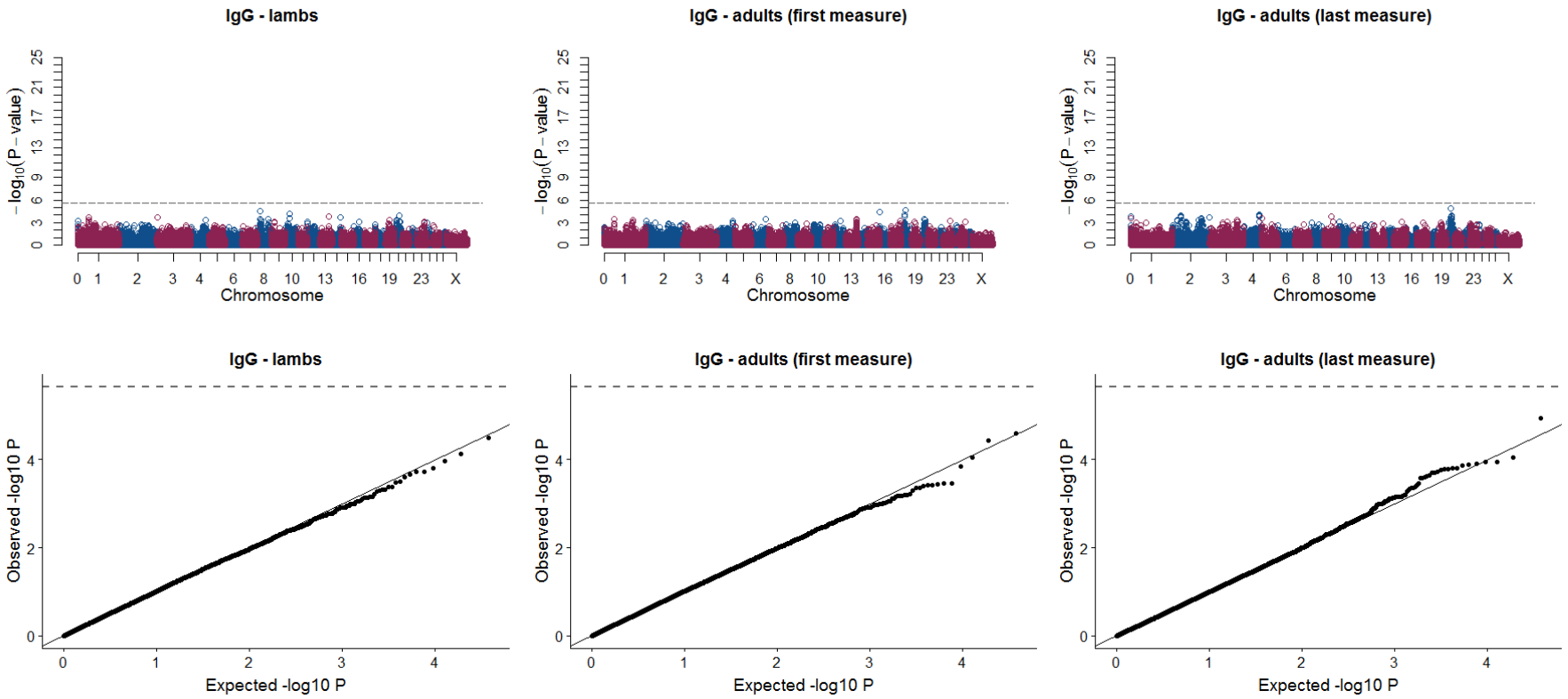

Figure 2.9. Genome-wide association results for anti-*T.circumcincta* IgG levels in Soay sheep in three different age classes (lambs, first adult measure, last adult measure). Manhattan plots of p-values are corrected for inflation (lambda) and the dashed line indicates the Bonferroni genome-wide significance threshold. Q-Q plots showing the association between expected and observed (lambda corrected) P-values. The solid line indicates the one to one line and the dashed line indicates the Bonferroni threshold. Inflation factors were > 1 in all cases.

Table 2.4. Genome-wide association results for anti-*T.circumcincta* IgA levels in Soay sheep. Genome-wide association was tested on three age groups (lambs, first measure as an adult and last measure as an adult). Included in the table are chromosome (Chr), number of observations (N), along with the p-values corrected for lambda (P). All SNPs included in the table are significant after correction for inflation (lambda) and are above the genome-wide significance threshold.

Trait	Age group	SNP	Chr	Position	Major allele	Minor allele	N	Additive effect	p
IgA	Lambs	s06827.1	0	0	G	A	2033	0.017 (0.002)	$1.28x10^{-6}$
		OAR24_11975271.1	24	10471141	G	A	2033	-0.027 (0.003)	$1.68x10^{-10}$
		OAR24_12006191.1	**24**	**10501046**	**G**	**A**	**2034**	**0.029 (0.002)**	**$8.88x10^{-19}$**
		s29806.1	24	10977878	A	G	2030	-0.039 (0.004)	$4.37x10^{-10}$
		s11845.1	24	11007102	A	G	2032	-0.030 (0.003)	$3.40x10^{-14}$
		s71593.1	24	11837275	C	A	2020	0.029 (0.003)	$5.43x10^{-9}$
		OAR24_13675640.1	24	12065734	A	G	2026	0.038 (0.005)	$1.90x10^{-6}$
	Adults (First measure)	s06827.1	0	0	G	A	1346	0.025 (0.003)	$1.05x10^{-8}$
		OAR24_8358348.1	24	7213312	A	G	1347	-0.030 (0.004)	$5.70x10^{-7}$
		s35996.1	24	8842899	G	A	1348	0.045 (0.006)	$5.88x10^{-7}$
		s33564.1	24	8874963	G	A	1347	0.045 (0.006)	$5.90x10^{-7}$
		s57548.1	24	9165564	G	A	1348	0.044 (0.006)	$1.17x10^{-6}$
		OAR24_10569180.1	24	9268996	G	A	1348	0.052 (0.007)	$6.40x10^{-8}$
		OAR24_11975271.1	24	10471141	G	A	1348	-0.045 (0.004)	$1.93x10^{-15}$
		OAR24_12006191.1	**24**	**10501046**	**G**	**A**	**1348**	**0.043 (0.003)**	**$2.59x10^{-25}$**
		s29806.1	24	10977878	A	G	1345	-0.068 (0.006)	$3.84x10^{-16}$
		s11845.1	24	11007102	A	G	1348	-0.050 (0.003)	$2.75x10^{-22}$
		s71593.1	24	11837275	C	A	1333	0.046 (0.004)	$4.71x10^{-13}$
		s61066.1	24	11883740	A	G	1348	-0.035 (0.005)	$1.72x10^{-6}$
		OAR24_13675640.1	24	12065734	A	G	1347	0.058 (0.007)	$7.09x10^{-9}$
	Adults (Last measure)	s06827.1	0	0	G	A	1345	0.025 (0.003)	$5.12x10^{-17}$
		OAR24_8358348.1	24	7213312	A	G	1346	-0.029 (0.004)	$2.91x10^{-13}$
		OAR24_10569180.1	24	9268996	G	A	1347	0.046 (0.006)	$8.22x10^{-13}$
		OAR24_11975271.1	24	10471141	G	A	1347	-0.042 (0.004)	$5.11x10^{-29}$
		OAR24_12006191.1	**24**	**10501046**	**G**	**A**	**1347**	**0.039 (0.003)**	**$1.11x10^{-46}$**
		s29806.1	24	10977878	A	G	1344	-0.063 (0.006)	$7.02x10^{-30}$
		s11845.1	24	11007102	A	G	1347	-0.046 (0.003)	$6.31x10^{-41}$
		s71593.1	24	11837275	C	A	1332	0.047 (0.004)	$4.25x10^{-28}$
		s61066.1	24	11883740	A	G	1347	-0.034 (0.005)	$4.11x10^{-12}$
		OAR24_13675640.1	24	12065734	A	G	1346	0.054 (0.007)	$4.38x10^{-16}$

2.4.4 SNP effect sizes

The GWAS can fit different fixed effects but only accounts for one variance component. Consequently, we fitted an animal model to obtain unbiased estimates of the effect sizes of the SNPs associated with anti-*T. circumcincta* IgA levels and to account for repeated measures. We took the 13 significant SNPs that were identified in the GWAS and included them as fixed effects as 3 level factors in an animal model using the complete dataset including all individuals in all age groups. All SNPs remained significantly associated with IgA levels in the animal model based on Wald tests (Table 2.5); all SNP associations had an additive effect on antibody levels, with the minor allele associated with increased IgA in 8 out of 13 SNPs (Figure 2.10). The difference in effects between homozygotes was considerable, ranging from 10 to 39% of the mean IgA level. Across all ages, each individual SNP explained 0.88 to 10.18% of phenotypic variation in IgA, corresponding to 2.45 to 28.19% of the additive genetic variance (Table 2.5). The observed associations with IgA are unlikely to be due to low frequency alleles, as all significant SNPs had a minor allele frequency >16.8%.

Table 2.5. Animal model results for the 13 significant SNPs found in the genome-wide association study run on the dataset across all ages on all sheep. Variance explained by each SNP, and the proportion of phenotypic and additive genetic variance explained was obtained by fitting each SNP as a factor in ASREML-R. Included are the estimate effect size of the heterozygous AB and homozygous BB where allele A increases the trait value (additive effect), the p-value (p) of the covariate in the animal model, variance explained by SNP (σ^2_{SNP}) and its associated standard error in parentheses, additive genetic variance (σ^2_A), heritability (h_2), proportion of additive genetic variance explained by the SNP ($\sigma^2_{SNP}/ \sigma^2_A$), the proportion of phenotypic variance explained by the SNP ($\sigma^2_{SNP}/ \sigma^2_P$) and the minor allele frequency (MAF).

Trait	SNP	Chr	Position	MAF	Effect AB	Effect BB	Mean trait value	p	σ^2_{SNP}	$\sigma^2_{SNP}/ \sigma^2_A$	$\sigma^2_{SNP}/ \sigma^2_P$
IgA	s06827.1	0	0	0.434	-0.111 (0.024)	-0.221 (0.029)	1.206	$2.23x10^{-13}$	0.006 (0.002)	0.060	0.022
	OAR24_8358348.1	24	7213312	0.329	-0.109 (0.019)	-0.194 (0.033)	1.206	$2.34x10^{-10}$	0.005 (0.001)	0.045	0.016
	s35996.1	24	8842899	0.200	-0.018 (0.043)	-0.123 (0.047)	1.206	$3.10x10^{-6}$	0.002 (0.001)	0.024	0.009
	s33564.1	24	8874963	0.200	-0.017 (0.043)	-0.122 (0.047)	1.206	$3.09x10^{-6}$	0.002 (0.001)	0.024	0.009
	s57548.1	24	9165564	0.197	-0.031 (0.044)	-0.136 (0.047)	1.206	$2.32x10^{-6}$	0.003 (0.001)	0.026	0.009
	OAR24_10569180.1	24	9268996	0.181	-0.065 (0.047)	-0.192 (0.050)	1.206	$6.76x10^{-9}$	0.004 (0.001)	0.040	0.014
	OAR24_11975271.1	24	10471141	0.358	-0.161 (0.019)	-0.351 (0.031)	1.206	$5.37x10^{-30}$	0.013 (0.003)	0.135	0.049
	OAR24_12006191.1	24	10501046	0.466	-0.232 (0.022)	-0.475 (0.027)	1.206	$4.16x10^{-64}$	0.028 (0.003)	0.282	0.102
	s29806.1	24	10977878	0.232	-0.198 (0.020)	-0.339 (0.040)	1.206	$1.33x10^{-27}$	0.012 (0.002)	0.122	0.044
	s11845.1	24	11007102	0.391	-0.228 (0.019)	-0.426 (0.029)	1.206	$3.30x10^{-49}$	0.022 (0.003)	0.223	0.080
	s71593.1	24	11837275	0.304	-0.121 (0.031)	-0.302 (0.035)	1.206	$6.49x10^{-25}$	0.009 (0.002)	0.089	0.032
	s61066.1	24	11883740	0.272	-0.087 (0.020)	-0.146 (0.037)	1.206	$4.79x10^{-6}$	0.003 (0.001)	0.025	0.009
	OAR24_13675640.1	24	12065734	0.168	-0.109 (0.051)	-0.267 (0.054)	1.206	$2.17x10^{-13}$	0.006 (0.002)	0.062	0.023

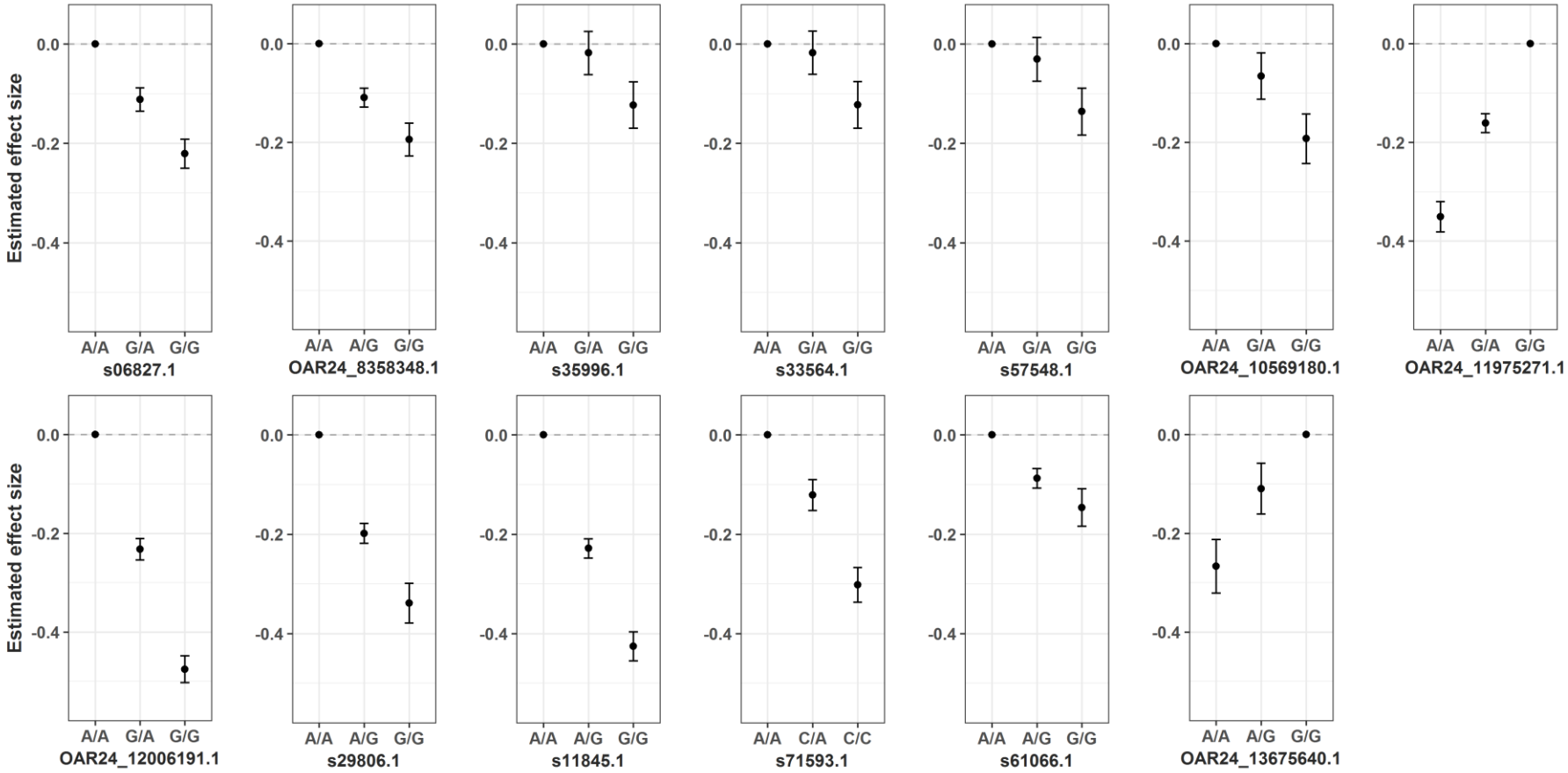

Figure 2.10. Anti-*Teladorsagia circumcincta* IgA levels and genotypes of 13 SNPs identified from the GWAS. Estimated effect sizes are from an animal model using the dataset across all ages on all sheep with effect sizes relative to the model intercept (set as the homozygote that increases the trait value). Error bars show the standard error of the mean. Units on the y-axis are corrected OD values as described in the methods.

2.4.5 Candidate genes

None of the 12 mapped SNPs on chromosome 24 were predicted to occur in genes or promoter regions, although OAR24_10569180.1 was an upstream variant (788 bp away) from epithelial membrane protein 2 (*EMP2*). Linkage disequilibrium was not high between the significant SNPs in this region (Figure 2.11). Of the loci in this region, the strongest candidate gene *a priori* was *CLEC16A. CLEC16A* has been associated with human common immunodeficiency and IgA deficiency (Ferreira *et al.* 2010; Li *et al.* 2015; Bronson *et al.* 2016) and *CLEC16A* knockdown mice have been associated with reduced number of B cells compared with controls (Li *et al.* 2015). However, *CLEC16A* is ~594 kb away from the most significant SNP (OAR24_12006191.1; Figure 2.11). It also does not appear that OAR24_12006191.1 is in high linkage disequilibrium with this region and SNPs closest to *CLEC16A* were not significant (Figure 2.12). OAR24_12006191.1 is in a region containing several poorly annotated and uncharacterised genes (Table 2.6). The nearest gene to the most significant SNP is uncharacterised on Ensembl (ENSOARG00000010696) but based on ontologies provided by Ensembl and UniProt is likely to be a membrane protein. This gene had no orthologues in humans or rodents, but had orthologues in the cow (*Bos Taurus*) and pig (*Sus scrofa*) genome.

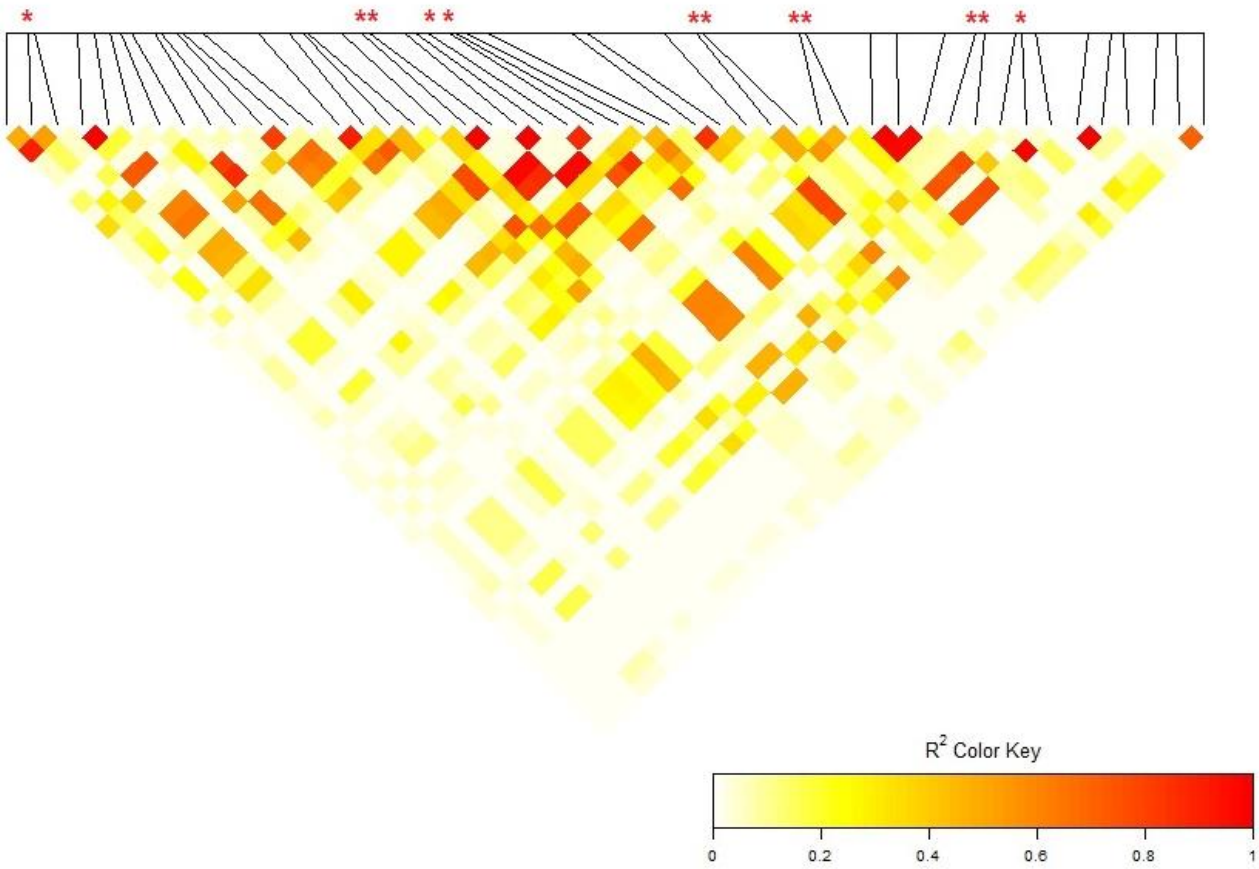

Figure 2.11. A heat map of the linkage disequilibrium (R^2) between all SNPs on chromosome 24 between 7 and 13 Mb created using the R library "LDheatmap" (Shin *et al.* 2006). Stars indicate the position of SNPs significantly associated with anti-*T. circumcincta* IgA levels from the GWAS run on first adult measures.

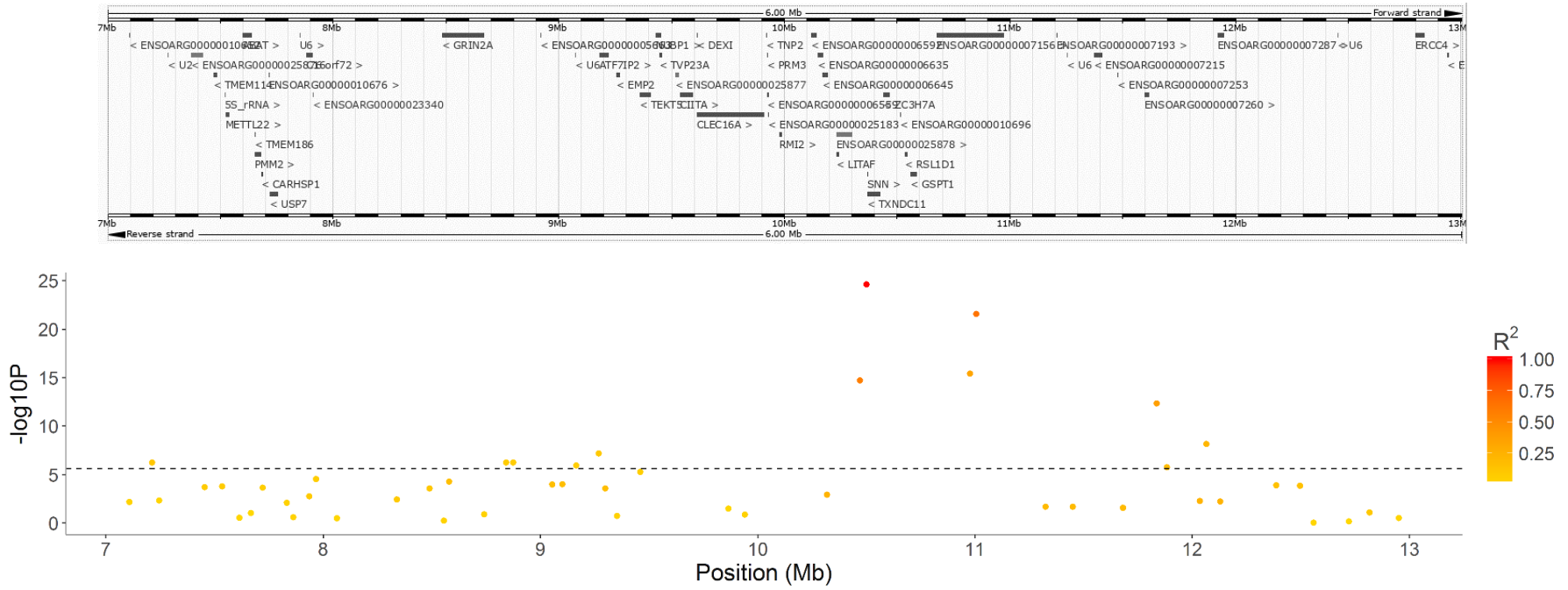

Figure 2.12. Associations between SNPs on chromosome 24 between 7 and 13Mb and anti-*T. circumcincta* IgA levels and the location of genes in this region. The top panel shows the genes in this region (from Oar_v3.1) as provided by Ensembl, while the bottom panel shows a Manhattan plot of p-values of SNPs from the GWAS run on first adult measures with SNPs colour coded for linkage disequilibrium (R^2) with the most significant SNP (OAR24_12006191.1).

Table 2.6. List of genes in the 7-13 Mb region of chromosome 24 from the *Ovis aries* genome reference v3.1 collated using Ensembl's BioMart.

Gene stable ID	Protein stable ID	Gene description	Gene start (bp)	Gene end (bp)
ENSOARG00000010652	ENSOARP00000011425		7097294	7097770
ENSOARG00000024293		U2 spliceosomal RNA	7269284	7269425
ENSOARG00000025876			7373003	7419823
ENSOARG00000005140	ENSOARP00000005508	transmembrane protein 114	7470804	7484932
ENSOARG00000024926		5S ribosomal RNA	7521851	7521935
ENSOARG00000005217	ENSOARP00000005600	methyltransferase like 22	7524698	7539289
ENSOARG00000005228	ENSOARP00000005615	4-aminobutyrate aminotransferase	7601608	7638449
ENSOARG00000010665	ENSOARP00000011439	transmembrane protein 186	7653053	7653562
ENSOARG00000005321	ENSOARP00000005710	phosphomannomutase 2	7655024	7679330
ENSOARG00000005359	ENSOARP00000005749	calcium regulated heat stable protein 1	7684641	7687775
ENSOARG00000010676	ENSOARP00000011457		7716171	7717367
ENSOARG00000005469	ENSOARP00000005875	ubiquitin specific peptidase 7	7721009	7754350
ENSOARG00000022733		U6 spliceosomal RNA	7854741	7854843
ENSOARG00000005554	ENSOARP00000005962	chromosome 16 open reading frame 72	7883148	7905643
ENSOARG00000023340			7912039	7912127
ENSOARG00000005625	ENSOARP00000006039	glutamate ionotropic receptor NMDA type subunit 2A	8485907	8665389
ENSOARG00000005663			8919198	8919567
ENSOARG00000023930		U6 spliceosomal RNA	9072610	9072716
ENSOARG00000005714	ENSOARP00000006127	activating transcription factor 7 interacting protein 2	9183296	9219109
ENSOARG00000005780	ENSOARP00000006199	epithelial membrane protein 2	9258704	9268208
ENSOARG00000005836	ENSOARP00000006262	tektin 5	9359214	9407395
ENSOARG00000005920	ENSOARP00000006345	nucleotide binding protein 1	9432662	9451103
ENSOARG00000006019	ENSOARP00000006447	trans-golgi network vesicle protein 23 homolog A	9448298	9456917
ENSOARG00000025877			9518636	9530140
ENSOARG00000006106	ENSOARP00000006543	class II major histocompatibility complex transactivator	9540624	9594737
ENSOARG00000006242	ENSOARP00000006683	Dexi homolog	9612662	9613128
ENSOARG00000006373	ENSOARP00000006833	C-type lectin domain containing 16A	9615768	9906837
ENSOARG00000006554	ENSOARP00000007016	transition protein 2	9919841	9921182
ENSOARG00000006556	ENSOARP00000007019	protamine 3	9925234	9925596
ENSOARG00000006559	ENSOARP00000007020		9927083	9928624

ENSOARG00000025183	ENSOARP00000022809		9931019	9931233
ENSOARG00000006567	ENSOARP00000007030	RecQ mediated genome instability 2	9980724	9986990
ENSOARG00000006592	ENSOARP00000007057		10120906	10142827
ENSOARG00000006635	ENSOARP00000007105		10151409	10169901
ENSOARG00000006645	ENSOARP00000007115		10170062	10190972
ENSOARG00000025878			10232183	10300719
ENSOARG00000006664	ENSOARP00000007132	lipopolysaccharide induced TNF factor	10233248	10241054
ENSOARG00000010684	ENSOARP00000011461	stannin	10368431	10368694
ENSOARG00000006723	ENSOARP00000007202	thioredoxin domain containing 11	10370778	10422595
ENSOARG00000006808	ENSOARP00000007295	zinc finger CCCH-type containing 7A	10438773	10464644
ENSOARG00000010696	ENSOARP00000011475		10515846	10516205
ENSOARG00000006923	ENSOARP00000007417	ribosomal L1 domain containing 1	10534085	10545779
ENSOARG00000007088	ENSOARP00000007603	G1 to S phase transition 1	10559022	10587328
ENSOARG00000007156	ENSOARP00000007673		10676205	10970881
ENSOARG00000007193	ENSOARP00000007707		11207107	11207326
ENSOARG00000023553		U6 spliceosomal RNA	11253051	11253157
ENSOARG00000007215	ENSOARP00000007731		11372442	11405761
ENSOARG00000007253	ENSOARP00000007772		11476498	11477487
ENSOARG00000007260	ENSOARP00000007777		11600441	11613855
ENSOARG00000007287	ENSOARP00000007804		11921141	11946628
ENSOARG00000023392		U6 spliceosomal RNA	12453544	12453651
ENSOARG00000007323	ENSOARP00000007853	ERCC excision repair 4, endonuclease catalytic subunit	12796810	12834021
ENSOARG00000007368	ENSOARP00000007899		12940773	12942020

2.5 Discussion

In this study, we investigated the genetic architecture underlying anti-*Teladorsagia circumcincta* antibody levels in a wild Soay sheep population. We have shown that these parasite-specific immune responses are highly repeatable, and this is in part due to genetic variation between individuals. Such high repeatability under natural conditions in a complex immune trait is notable because of the highly variable environment individuals experience on St Kilda. The different isotypes were not strongly correlated, suggesting that individual sheep may develop distinct antibody phenotypes which are temporally very stable despite marked annual variation in exposure to nematode parasites, food availability and climate conditions (Coulson *et al.* 2001; Crawley *et al.* 2004; Wilson *et al.* 2004). We also found that there was a large jump in anti-*Teladorsagia circumcincta* antibody levels between lambs and yearlings which has previously been observed in the Soay sheep and may be due to the development of anti-helminth immunity with exposure over early life (Coltman *et al.* 2001b). These antibody levels were also moderately heritable, suggesting that they have the potential to respond to selection. Finally, we identified a QTL on chromosome 24 in which a single SNP explained 28% of the additive genetic variance for parasite-specific IgA levels. This study provides a rare example of a particular genomic region explaining variation in an immune measure in the wild, and provides evidence for large effects of variation in non-MHC regions of the genome on immune phenotype, as well as providing a new region for exploration for candidate loci.

In adults, levels of the three different antibodies were highly repeatable within sheep yet largely uncorrelated, suggestive of complex individualised immune phenotypes which are consistent over lifetimes. Repeatabilities of anti-*T. circumcincta* IgG levels were comparable to those previously reported with an anti-*Teladorsagia cirumcincta* pan-isotype (largely measuring IgG) antibody measure, while repeatabilities of anti-Tc IgA and IgE levels across all ages were also comparable to other morphometric traits in the Soay sheep such as body weight (Hayward *et al.* 2014). Longer term longitudinal immunology studies in humans are beginning to emerge, motivated by the importance of immune variation in driving altered efficacy of vaccinations, as well as impacts on susceptibility to a range of diseases that vary throughout life including allergies, infectious disease, autoimmunity and inflammatory diseases (Liston *et al.* 2016). Our results appear broadly consistent with the consensus

emerging from these studies, which have also determined that the extensive variation in immune parameters in human populations is driven by high inter-individual variability and low intra-individual variation, indicative of stable immunological profiles of individuals (Tsang *et al.* 2014; Carr *et al.* 2016). The relatively small amount of variation explained by cohort, maternal and annual effects found here suggests that temporal variation in exposure to parasites, condition or early life effects had relatively little influence on antibody levels. Together, these results are indicative of the stable individual immune phenotypes observed in human studies (Liston *et al.* 2016). We also found a considerable permanent environment effect, accounting for 39%, 63% and 58% of the repeatability across all ages for IgA, IgE and IgG respectively. This permanent environment component could be driven by numerous factors including consistent spatial differences in exposure or individual disease history, or due to complex interactions between nutritional state, exposure, other parasites and life history during early life. Our unique longitudinal dataset points to individualised but highly consistent antibody responses to helminths across the lifetime of a long-lived wild mammal.

There was a significant heritable component to all three antibody traits across all age groups tested. Previous work in the Soays using an anti-*Teladorsagia cirumcincta* pan-isotype antibody measure (that probably primarily measured IgG levels) found similar estimates for heritability in lambs (Brown *et al.* 2013) and across all ages (Hayward *et al.* 2014). Previous analyses from domestic sheep have given similar heritability estimates of anti-*T. circumcincta* IgE of 0.39±0.16 against third stage larvae and 0.50±0.16 against fourth stage larvae in Texel lambs (Murphy *et al.* 2010) while heritability of anti-*T.circumcincta* IgA was 0.56±0.11 against fourth stage larvae in Scottish Blackface lambs (Strain *et al.* 2002). A significant heritable component to a number of immune parameters has also been observed in human populations (Evans *et al.* 1999; Orrù *et al.* 2013; Ye *et al.* 2014; Brodin *et al.* 2015; Roederer *et al.* 2015). However, heritabilities appeared to increase from lambs to adults for two antibody isotypes in this study, which contrasts with a previous study in humans that found that monozygotic twins became more divergent in their immune profiles with increasing age (Brodin *et al.* 2015). Estimating the phenotypic and genetic correlations of antibody levels between lambs and adults in our study would improve our understanding of how the genetic basis of these traits may differ in early and adult life. While studies in wild birds do not consistently find a heritable component to the PHA response or haematocrit levels (Pitala *et al.* 2007; Bonneaud *et al.* 2009; Morrison *et al.* 2009; Drobniak *et al.* 2010; Kim *et al.* 2013; Sakaluk *et al.* 2014), heritability estimates in wild mammals are

rare (Graham *et al.* 2010; Hayward *et al.* 2014). Our results support those carried out in humans, domestic sheep and wild vertebrates that have documented considerable genetic variation driving immune responses. Strong selection on a trait is likely to reduce underlying genetic variation and hence heritability of quantitative traits (Falconer & Mackay 1996). The significant heritability seen in immune variation in our study as well as in natural populations may be in accordance with current theory which predicts that stabilising selection rather than directional selection is likely acting on immune traits (Seppälä 2015). Interestingly, IgG appeared to have a lower heritability, which may be due to the positive association between IgG levels and over-winter survival, an association that was not observed with IgA or IgE (Nussey *et al.* 2014; Watson *et al.* 2016).

This study has documented the first GWAS on an immune phenotype in a wild population, and has shown evidence for non-MHC regions having a large effect on these traits. We have also found evidence for differences in the genetic architecture underlying the three different immune traits. Anti-*T. circumcincta* IgE and IgG levels were not significantly associated with any SNPs in the GWAS, suggesting that they are classic polygenic traits influenced by many small effect genes across the genome. The lack of identification of QTL associated with IgG is supported by a previous study in the Soay sheep using an anti-*T. circumcincta* pan-isotype antibody measure which did not find significant QTL using a candidate gene approach (Brown *et al.* 2013). In contrast, IgA levels were associated with multiple SNPs marking a major QTL on chromosome 24. Each individual SNP explained between 2.45 and 28.19% of the additive genetic and 0.88 to 10.18% of the phenotypic variance. This is in contrast with a number of GWAS studies in the wild which have found few, if any, associations of SNPs with complex phenotypic traits (Johnston *et al.* 2014; Bérénos *et al.* 2015; Husby *et al.* 2015; Santure *et al.* 2015; Wenzel *et al.* 2015; Kardos *et al.* 2016; Silva *et al.* 2017). Our study provides evidence that high genetic variance and major effect QTLs underlying immune responses can persist despite varying environmental conditions and natural selection in the wild.

Within the QTL region on chromosome 24, it is unclear which actual gene or genes are influencing IgA levels. The region containing significant SNPs equates to 5 Mb, and none of the genotyped SNPs are in promoter or coding regions. SNPs across this region did not all appear to be in high linkage disequilibrium with each other and it is uncertain whether there is one or more causative loci in this region. The most significant SNP with the largest effect

size, OAR24_12006191.1, was in a relatively poorly annotated region with a number of uncharacterised genes. The strongest candidate gene in this region is *CLEC16A*, which has recently been associated with common variable immunodeficiency disorder, characterised by inadequate antibody responses, and IgA deficiency (Ferreira *et al.* 2010; Li *et al.* 2015; Bronson *et al.* 2016). *CLEC16A* knockdown mice have a reduced number of B cells and increased IgM levels compared with controls (Li *et al.* 2015). Yet, despite this being a strong candidate gene in this region *a priori*, this gene is ~594 kb away from the most significant SNP. Additionally, in the Soay sheep this SNP did not appear to be in high linkage disequilibrium with SNPs closest to the gene, and SNPs closest to the gene were not significantly associated with IgA. Further study into this region using the high density SNP chip, sequencing or expression data is warranted to identify the candidate gene. Sequence information would provide a better idea of potential causal mutations and identify whether they are in regulatory or protein-coding regions, while expression data would allow us to investigate whether these genetic variants would affect CLEC16A gene or protein expression.

To the best of our knowledge, no previous candidate gene or genome-wide analyses in sheep have documented a QTL associated with anti-*T. circumcincta* IgA levels on chromosome 24. Potentially causal loci have been identified on chromosome 3 in Soay and Scottish blackface lambs and chromosome 20 in Scottish blackface lambs, corresponding to the IFNγ gene and the MHC locus respectively, using candidate gene and QTL mapping (Coltman *et al.* 2001b; Davies *et al.* 2006). A later QTL study in adult Spanish churra ewes found a locus on chromosome 1 (Gutiérrez-Gil *et al.* 2009). We could find only two previous genome wide association studies on anti-*T. circumcincta* IgA levels using the ovine 50K SNP array. One study in Scottish Blackface lambs failed to identify any SNPs associated with *T. circumcincta* IgA (Riggio *et al.* 2013), while the other study in Spanish churra ewes found one genome-wide significant SNP on chromosome 12 and nine additional chromosome-wise significant SNPs on chromosomes 8, 10, 11, 14, 15, and 25 (Atlija *et al.* 2016). A further QTL mapping study found total IgE and anti-*Trichostrongylus colubiformis* IgG levels were each associated with a region on chromosome 23 in Romney lambs (Crawford *et al.* 2006). The MHC region is not assessed on the ovine 50K SNP array due to the high level of variation within this region and low density of SNPs around this region which may explain the lack of identification of this region in our study and previous GWAS studies in sheep. Together with our results, it appears that QTL for parasite-specific antibody traits have not

been consistently observed between sheep breeds, which may be due to different loci associated with immune responses in the different ages looked at, differences in host-parasite exposure, or inherent differences between breeds (Brown *et al.* 2013; Atlija *et al.* 2016). The lack of consistency with previous studies in sheep may be due to loci underlying immune responses to parasites not being conserved between sheep breeds, due to different breeds having been subjected to different selective breeding histories and genetic drift (Kijas *et al.* 2012). The identification of this QTL may also be due to a genotype-by-environment effect that may only be manifested under natural conditions or could have been introduced with a historical admixture event with the Dunface breed (Feulner *et al.* 2013). Identification of the candidate gene influencing IgA levels, and investigating the selection on SNP genotypes and haplotypes in Soay sheep, is required to fully understand why this region has not been previously identified in domestic sheep.

2.6 Conclusion

We have shown that anti-*T. circumcincta* antibody levels show a highly distinctive but temporally stable pattern of variation over the adult lifetime of a long-lived wild mammal, despite variable and challenging environmental conditions. Antibody levels were moderately heritable in both 4-month lambs and adults and are a potential large target for contemporary selection. Further study will be needed to shed light on why these immune traits are so heritable, by using sequence information to look at signatures of selection and associations with fitness data. The three antibody isotypes differ in their underlying genetic architecture, with IgE and IgG under polygenic control, while for IgA, one genome region of large effect has been identified. We have shown that a complex immunological trait can be under strong genetic control in the wild, with small genomic regions explaining a considerable proportion of the additive genetic variance. This provides evidence that high additive genetic variation and major effect QTLs underlying immune traits can be present despite huge variability in environmental conditions and natural selection in the wild. This study provides the first evidence from a genome-wide study that large effect genes outside the MHC region exist for immune traits in nature, and highlights the importance of further study of this region to understand why it has been identified in a wild, but not in domestic, sheep studies

Chapter 3

Natural selection on anti-helminth antibody levels in a wild mammal population

3.1 Summary

Individuals in wild populations are constantly challenged by micro- and macroparasites, which have negative effects on host fitness. The immune system is critical in the defence against parasites, and effective immune responses to infection are therefore expected to be under positive selection. However, immune responses incur energetic and physiological costs resulting in trade-offs between protection against parasites and other fitness components such as reproduction. Furthermore, the costs of mounting an immune response and the pattern of selection on immunity are expected to vary depending on host sex and age, environmental exposure to the parasite, as well as on the aspects of immunity being measured. This is likely to generate complex, potentially stabilising selection on immune phenotypes in natural systems. Whilst an increasing number of wild vertebrate studies find positive associations between immune response phenotypes and survival and negative associations with reproductive traits, comprehensive assessments of sex-, age- and environment-dependent selection on ecologically-relevant immune phenotypes are lacking. Here, we measured three functionally distinct isotypes (IgA, IgE and IgG) of antibodies against a prevalent strongyle nematode parasite (*Teladorsagia circumcincta)* in wild Soay sheep over a 25-year period. We tested for relationships among these antibody isotypes and host body weight, strongyle faecal egg count, over-winter survival and subsequent fecundity and for interactions between antibody levels and sex, age and host population size. Associations between strongyle faecal egg count and antibody levels were age- and isotype-dependent: low faecal egg counts were predicted by high anti-*T. circumcincta* IgA levels in lambs but high IgG levels in adults. Associations between antibody levels and weight were

generally positive but rarely significant or strong, suggesting variation in the immune phenotypes was unlikely to simply reflect variation in host condition. We found evidence of positive directional selection but this, again, was isotype-, age- and sex-dependent: high anti-*T. circumcincta* IgG levels predicted increased over-winter survival probability in adult females and subsequent fecundity in adult males, with little evidence of selection on the other two isotypes or in lambs. Our results highlight the complexity of natural selection on immune phenotypes in natural systems, and suggest that patterns of selection are unlikely to generalise across different immune traits or host demographic groups in the wild.

3.2 Introduction

Parasites have a major impact on host condition and fitness and thereby represent a strong selective force for individuals in natural populations (Schmid-Hempel 2011). The main defence against parasites is the host immune system (Murphy 2012), and consequently it is expected for there to be a strong directional selection in favour of robust and effective immune responses in natural populations (Nunn, Gittleman & Antonovics 2000; Lindström *et al.* 2004; Scharsack *et al.* 2007). Directional selection on a phenotypic trait is predicted to erode genetic variation in that trait (Falconer & Mackay 1996), but considerable genetic variation underlying parasite burden, infection risk and immune phenotypes has been observed in humans, laboratory studies and in the wild (Lazzaro & Little 2009; Maizels & Nussey 2013). A key explanation for the maintenance of genetic variation in immune responses in the face of selection lies in the fact that mounting an immune response can incur considerable energetic costs (Lochmiller & Deerenberg 2000). Although investing in immunity may promote survival in the face of parasite infection, it draws resources away from other key fitness functions such as growth and reproduction (Sheldon & Verhulst 1996). Alternatively, mounting strong immune responses can lead to damage to host tissue and autoimmunity which would impose a fitness cost (Viney *et al.* 2005; Graham *et al.* 2005). These processes could result in stabilising, rather than directional selection, on immune phenotypes (Seppälä 2015).

Importantly, both the costs of investing in immune responses and the pattern of selection on immune traits are expected to vary with environment, sex and age (Seppälä 2015). Parasite exposure risk is expected to vary over space and time, and the fitness cost to benefit balance

of investing in immunity to that parasite will therefore also vary (Altizer *et al.* 2006). Similarly, heightened intrasexual competition and immunosuppressive effects of testosterone experienced by males, particularly in polygynous species, leads to the expectation of differences in the fitness cost and benefits of investing in immunity between the sexes (Zuk 1990). Both immune phenotype and the ability to mount an effective response to infection also vary profoundly with host age (Palacios *et al.* 2011; Simon *et al.* 2015). In particular, young immunologically naïve hosts tend to have the highest infection rates and parasite burdens, and the fitness costs and benefits of investing resources in immunity during early life may differ profoundly from those in adulthood (Jackson *et al.* 2014). Given the staggering complexity of the vertebrate immune system, it is also important to consider that the pattern of natural selection may also vary considerably across different components of the immune response and depend hugely on the immune assays chosen in a particular study (e.g. Gonzalez *et al.* 1999; Råberg & Stjernman 2003; Parejo & Silva 2009; Nussey *et al.* 2014; Watson *et al.* 2016). For instance, a general measure of response to a non-specific challenge, such as the swelling response to phytohaemagglutinin challenge widely used in eco-immunology, may be informative about selection on the host's general capacity to respond to generic immune challenge but not necessarily reflect how selection operates on specific immune responses to ecologically-relevant infectious agents (Graham *et al.* 2011; Demas *et al.* 2011).

In natural populations, variation in resource acquisition will influence the total availability of resources for investment in immunity and other life history traits (Siva-Jothy & Thompson 2002; Seppälä & Jokela 2010). This may generate positive associations between immune traits and fitness components due to differences in host condition and resource acquisition and obscure trade-offs between immunity and life history traits (van Noordwijk & de Jong 1986; Kraaijeveld & Godfray 1997; Reznick, Nunney & Tessier 2000). Despite this, studies in natural vertebrate populations do provide support for the hypothesis that higher immune responses predict improved survival (Saino *et al.* 1997; Christe *et al.* 1998, 2001; Merino *et al.* 2000; Nussey *et al.* 2014; Watson *et al.* 2016), but reduced reproductive performance (Ilmonen *et al.* 2000; Råberg *et al.* 2000; Bonneaud *et al.* 2003; Uller, Isaksson & Olsson 2006; Marzal *et al.* 2007; Gasparini *et al.* 2009; Graham *et al.* 2010; Hayward *et al.* 2014). However, there is also emerging evidence that selection on immune measurements may be environment-dependent (Svensson, Sinervo & Comendant 2001; Calsbeek, Bonneaud & Smith 2008; Graham *et al.* 2010) and that patterns of selection may vary among immune

measures (Gonzalez *et al.* 1999; Parejo & Silva 2009; Nussey *et al.* 2014; Watson *et al.* 2016). For example, antibody responses of wild blue tits to diphtheria and tetanus antigens showed positive linear and curvilinear relationships, respectively, with over-winter survival (Råberg & Stjernman 2003). To date, no study has comprehensively tested for sex-, environment- and age-dependent associations between immune measurements and subsequent survival and fecundity, whilst correcting for host physiological condition, in a wild vertebrate.

Here, we examine the relationships among three functionally distinct isotypes (IgA, IgE and IgG) of anti-parasite antibodies and strongyle faecal egg count, host body weight, survival and fecundity in a free-living Soay sheep population. Previous studies of this system have demonstrated strongly environment-, age- and sex-dependence of natural selection on traits such as body weight and horn growth (Robinson *et al.* 2006, 2008; Wilson *et al.* 2007). In addition, there is evidence for survival costs of reproduction for young and old females, but only during severe environmental conditions (Tavecchia *et al.* 2005). The Soay sheep are infected with a number of parasitic helminths, including a variety of gastrointestinal strongyle nematodes, largely comprised of the species *Teladorsagia circumcincta, Trichostrongylus axei* and *Trichostrongylus vitrinus* (Wilson *et al.* 2004; Craig *et al.* 2006). Strongyle nematode burden, in combination with harsh winter climates and low food availability, are a strong selective force on the sheep (Gulland 1992; Wilson *et al.* 2004; Craig *et al.* 2006; Hayward *et al.* 2011). An exhaustive examination of both directional and stabilising selection on strongyle faecal egg counts (FEC), found evidence of age- and environment-dependencies: high FEC predicted reduced survival but only in lambs when population size was small (Hayward *et al.* 2011). Immunological research on domestic sheep and laboratory mice demonstrates that antibodies, particularly those of the functionally distinct isotypes IgA, IgE and IgG, play an important role in both the development and maintenance of immunity to these parasites (Stear *et al.* 1995; Blackwell & Else 2001; Strain *et al.* 2002; Gurish *et al.* 2004; McCoy *et al.* 2008; Murphy *et al.* 2010). In cross-sectional samples collected in one or a few years, we have previously assayed antibodies of these isotypes against antigens from larval stages of *T. circumcincta*. We detected highly isotype-specific relationships with body weight and FEC and positive associations between anti-*T. circumcincta* IgG antibody levels and subsequent survival (Nussey *et al.* 2014; Watson *et al.* 2016). A larger-scale study, spanning 11 years, found that anti-*T. circumcincta* pan-isotype antibody levels were associated with reduced breeding success in the subsequent year for

both adult males and females of high body weight (Hayward *et al.* 2014). This suggests that variation in immune defences in Soay sheep may be maintained by opposing selection on survival and fecundity, and that selection may be sex- and environment-dependent.

In this chapter, we test the associations of anti-*T. circumcincta* IgA, IgE, IgG antibody levels with strongyle FEC and weight, and elucidate the patterns of selection acting on antibody levels in a 25-year dataset. In the preceding chapter, we found that antibody levels were significantly heritable in all ages, and that IgA levels were significantly associated with genetic variants in a region on chromosome 24 (Chapter 2). In this chapter, we test associations between antibody levels and strongyle FEC to investigate whether levels are indicative of exposure (positive associations) or resistance (negative associations). Next, we investigated whether antibody levels were associated with condition by looking at relationships with August weight. If there were strong positive associations between immune measures and August weight, we might expect this to be due to overall condition of the individual. Selection analyses were performed using two measures of fitness, annual breeding success and over-winter survival in the year following the measurement. We tested for both linear and quadratic associations of antibody levels with each trait to investigate the presence of directional and stabilising selection respectively. Crucially, we included weight in all selection analyses in order to account for some of the confounding effects of condition, and to uncover whether we could observe life history trade-offs with immunity. Next, we investigated whether these associations were age, sex- or density- dependent. We might expect for there to be a higher cost of immunity in males compared to females and stronger selection in lambs and at high population densities due to the higher risk of parasitism (Wilson *et al.* 2004; Hayward *et al.* 2014). Finally, we looked at whether we could observe selection on 13 SNPs that were previously identified to be associated with anti-*T. circumcincta* IgA levels (Chapter 2). We would expect either weak or balancing selection to be occurring on these SNPs in order for this level of variation to be maintained.

3.3 Methods

3.3.1 Study population

Soay sheep are a primitive breed of domestic sheep that were isolated on the island of Soay in the remote St Kilda archipelago several millennia ago, and have been living under unmanaged conditions and evolving under natural selection since then (Clutton-Brock & Pemberton 2004). In 1932, just over 100 Soay sheep were moved to the larger island of Hirta after the evacuation of all human residents. Approximately a third of the population of these sheep live in the Village Bay area of Hirta, and these individuals have been the subject of a long term study since 1985 (Clutton-Brock & Pemberton 2004). In April each year around 95% of all lambs born in this area are caught and individually tagged. Each August, as many sheep as possible from the study population are re-captured using temporary traps (Clutton-Brock & Pemberton 2004). At capture, animals are weighed and blood and faecal samples are collected. Whole blood samples are collected into heparin tubes, centrifuged at 3000 r.p.m. for 10 minutes, and plasma removed and stored at -20°C. Strongyle faecal egg count (FEC) is estimated from faecal samples as the number of eggs per gram using a modified McMaster technique (Gulland & Fox 1992). Contributing to the majority of the strongyle FEC are three species: *T. circumcincta*, *Trichostrongylus axei* and *Trichostrongylus vitrinus.* (Craig *et al.* 2006). The majority of sheep deaths occur over winter, and the population dynamics of the Soay sheep are characterised by periods of low but rising population sizes followed by high mortality 'crash' winters in which over half the population can die (Clutton-Brock & Pemberton 2004). Regular censuses and mortality searches during the winter months result in the majority of carcasses being discovered and provide accurate death date information.

The number of offspring from individuals in the study population is well known and annual breeding success is calculated differently depending on sex. For female annual breeding success, we used the number of offspring born to the ewe in the subsequent spring (derived from observational data), while for males we calculated the number of offspring sired by the ram and born in the subsequent spring (derived using the pedigree). The pedigree used was constructed using maternities and paternities assigned with 315 unlinked single nucleotide polymorphisms (linkage disequilibrium $r^2 < 0.05$) with a minor allele frequency > 0.4 using

the R library *sequoia* (Huisman 2017). Total Village Bay population (referred to as density subsequently) was used as a crude measure of environmental conditions, as it captures variation in both food availability and nematode parasite exposure (Wilson *et al.* 2004; Hayward *et al.* 2014).

In this analysis we included all animals that were caught and subsequently had plasma samples taken in August between 1990 and 2015. This encompassed 6543 samples from 3190 individuals over the 25-year period. Five samples from late-born lambs caught in August that were within 50 days of birth were excluded from the dataset, due to the potential presence of maternal antibodies and differences in development stage to other lambs.

3.3.2 Laboratory methods

IgA, IgG and IgE activity against antigens of the third larval stage of *T. circumcincta* were measured using direct (IgA, IgG) and indirect (IgE) ELISAs (henceforth, "anti-Tc antibodies"). We used *T. circumcincta* L3 somatic antigen, provided by the Moredun Research Institute, as the capture antigen for both assays diluted to 2µg/ml in 0.06M Carbonate buffer at pH 9.6. 50µl of the diluted capture antigen was added to each well of a Nunc-immuno 96-microwell plate, which was covered and incubated at 4°C overnight. After washing the wells three times in Tris-buffered saline-Tween (TBST) using a plate washer, 50µl of the Soay sheep plasma sample diluted to 1:50 for IgA and IgE, and 1:12800 for IgG was added to each well. The plates were then covered and incubated at 37°C for 1 hour. Plates were then washed five times with TBST and 50µl per well of rabbit anti-sheep IgA detection antibody conjugated to horseradish peroxidase (HRP) (AbD Serotec AHP949P) diluted 1:16000 was added to the anti-*T. circumcincta* IgA assay and 50µl per well of rabbit anti-sheep IgG detection antibody conjugated to HRP (AbD Serotec 5184-2104) diluted 1:16000 was added to the anti-*T. circumcincta* IgG assay. For the anti-*T. circumcincta* IgE assay, 50µl per well of anti-sheep IgE (mouse monoclonal IgG1, clone 2F1, provided by the Moredun Research Institute) diluted 1:100 was added, followed by 1 hour incubation at 37°C, five washes with TBST and then 50µl per well of goat anti-mouse IgG1-HRP detection antibody (AbD Serotec STAR132P) was added diluted to 1:8000 in TBST. All plates were then incubated at 37°C for 1 hour. Plates were then washed five times with TBST, and 100µl of SureBlue TMB 1-Component microwell peroxidase substrate (KPL) was added per well and left to incubate for 5 minutes in the dark at 37°C. Reactions were

stopped by adding 100µl per well of 1M hydrochloric acid and optical densities (OD) were read immediately at 450nm using a Thermo Scientific GO Spectrophotometer.

All results were recorded as OD values. In order to minimise confounding of capture year and age effects with plate to plate variation, each plate included samples from two years paired at random with different age groups on each plate. All plates were run in duplicate and duplicate sample ODs were removed if the coefficient of variation was > 0.2 and the difference between ODs was greater than 0.2. We also checked the correlation of ODs across duplicate plates and re-ran both plates if $r < 0.8$. To reduce error due to within-plate variation, we included, per plate, two sample free wells (50µl TBST) as blanks and two wells of positive controls. Positive controls for the IgE assay were serum from ewes trickle infected with *T. circumcincta*, and for the IgA and IgG assay were plasma from normal healthy non-immunised domestic sheep. For subsequent analyses, the mean optical density ratio of each sample was taken according to the formula:

$$OD = \frac{(\text{sample OD} - \text{blank OD})}{(\text{positive control OD} - \text{blank OD})}$$

Where the numerator was set to zero if the blank OD was greater than the sample OD (in order to avoid negative values). The number of samples that failed quality control per assay was 13 for IgA (7 lambs and 6 adults), 8 for IgE (6 lambs and 2 adults) and 27 for IgG (5 lambs and 22 adults).

3.3.3 Statistical analyses

All analyses were conducted using linear and generalised linear mixed models in R v3.3.3. Due to the immaturity of the immune system at 4 months, and the large increase in antibody levels between 4 and 16 months (see Chapter 2), we ran three models per response variable – for lambs (both sexes), adult females and adult males (where adults are sheep ≥ 12 months old). Adults were separated into females and males due to the differences in range and distribution of phenotypic traits such as weight and FEC in adults (Figure 3.1). We built models with August weight, August strongyle FEC, over-winter survival and annual

breeding success as response variables with anti-*T. circumcincta* IgA, IgE and IgG levels as explanatory variables. Continuous variables were rescaled to mean 0 and standard deviation 1 prior to inclusion in each model subset. We first fitted a model containing the fixed effects previously identified as important predictors of these variables, as described below. We then simplified the model by step-wise deletion, sequentially removed fixed effects with the lowest t values and determined statistical significance using likelihood ratio tests until a base model containing only significant ($p < 0.05$) fixed effects was left. All dropped terms were then re-tested against this base model using the same criteria. Population size and sex (where fitted) were kept in all models, in order to test for interactions with antibody levels. To the minimal model containing only significant terms we separately added linear and quadratic functions of anti-Tc IgA, IgE and IgG, and assessed them for significance using likelihood ratio tests. Next we tested whether there were sex- or density-dependent effects of antibody levels in each of the models by looking at interactions with sex and population density against the linear and, if significant, quadratic antibody terms. Finally, we added the 13 significant SNPs associated with anti-Tc IgA levels in Chapter 2 to the minimal models for our fitness measures of over-winter survival and breeding success.

August strongyle FEC was heavily right-skewed and zero-inflated. Since the accuracy of the method used to estimate FEC is limited to 100 eggs per gram (Gulland & Fox 1992), we binned August FEC into multiples of 100, with 0s counted as 0, and values > 60,000 eggs grouped into a single final bin (Figure 3.1). Associations between antibody measures and August strongyle faecal egg count (FEC) were modelled using generalised linear mixed models via the "glmmADMB" package. To assess which distribution best fitted the dataset, we tested Poisson distributions and two negative binomial distributions ("NB2" parameterization where variance = $\mu(1 + \mu/k)$ and "NB1" fit where variance = $\varphi\mu$) with and without zero-inflation, and compared the AIC between all six models. We chose the best fit model based on AIC values, where the model with the lowest value was selected, unless it was different by less than 2 AIC values from a model with fewer parameters. The model with the lowest AIC value was the negative binomial "NB2" distribution without zero-inflation for all models: lambs, adult females and adult males. For lambs, we included sex, twin status, weight (linear and quadratic), age in days (linear and quadratic) and population density as fixed effects and included year as a random effect. For the adult models, we included age (linear and quadratic), weight (linear and quadratic) and population size as fixed effects, and individual identity and capture year as random effects.

We investigated potential associations between August weight and anti-Tc antibody levels using linear mixed effects models via the "lme4" package, assuming a Gaussian distribution (Figure 3.1). For the lamb models we included sex, twin status, age in days, maternal age (quadratic) and population size as fixed effects, and capture year and maternal identity as random effects. For adult weight models, we included age (linear and quadratic terms) and population size as fixed effects, and individual identity and year as random effects.

We calculated over-winter survival as sheep that survived to 1st May in the subsequent year, using death date information, capture and census information. Survival probability varied with age (Figure 3.2). In lambs, on average 50% of females and 40% of males in our models survived their first winter, whilst in adults 88% of females and 72% of males survived the winter subsequent to antibody measurement. Analyses of survival were performed using generalised linear mixed models (GLMMs) in the "lme4" package with a binomial error structure. Binomial GLMMs were run with the built-in "bobyqa" optimiser and the maximum number of iterations increased to $2e^{-6}$ to improve model convergence. For the lamb model we included sex, twin status, weight (linear and quadratic), population size and interactions between sex and weight (linear and quadratic) as fixed effects, and capture year as a random effect. For the adult models we included age (linear and quadratic), weight (linear and quadratic) and population size as fixed effects, with capture year as a random effect.

For female annual breeding success, we used the number of offspring born to the ewe in the subsequent spring from observational data, excluding all individuals that did not survive the winter. For male annual breeding success, we calculated the number of offspring sired by the ram and born in the subsequent spring using the pedigree. The male annual breeding success measure included males that died over the winter, but excluded all males that were not seen to participate in the rut in the census records taken between October and December. In lambs, 44% of females had a lamb, and 9% of males sired a lamb in their first year, whilst in adults 87% of females and 51% of males had a lamb the subsequent year. Since the distributions of fecundity measures are very different for males and females, sex-specific models were run for breeding success in both lambs and adults. Female annual breeding success measures were treated as binary in both lambs and adults (lambed/did not lamb), while male fecundity measures were treated as binary only in lambs due to the low number of individuals siring lambs (9%) in their first year and all three models were run as a GLMM

with a binomial error structure as for survival. For adult males, we analysed annual breeding success in the "glmmADMB" package, using the same model selection process described above for the strongyle FEC models. The best fit model was the negative binomial with the "NB2" parameterisation, without zero-inflation. For female lamb models, we included twin status, weight (linear and quadratic) and population size as fixed effects, and individual identity as a random effect. The male lamb model had a similar model structure but included horn type (factor: normal horns or scurred) as an additional fixed effect. For female adult breeding success we included age (linear and quadratic), weight (linear and quadratic) and population size as fixed effects, and individual identity and year as random effects. The male adult breeding success model had a similar model structure to female adult breeding success but included horn type as an additional fixed effect.

Due to the high proportions of females breeding as adults, we also ran models of annual reproductive success in adult females. This measure was calculated as whether a ewe had any lambs surviving to August the following year, using death date and census information, for all ewes that had given birth to a lamb that year. Female annual reproductive success measures were treated as binary (surviving lambs/no surviving lambs) and were run as a GLMM with a binomial error structure. 86% of females that gave birth had a lamb that survived to August in the subsequent year. In this model, we included age (linear and quadratic), weight (linear and quadratic) and population size as fixed effects, and individual identity and year as random effects.

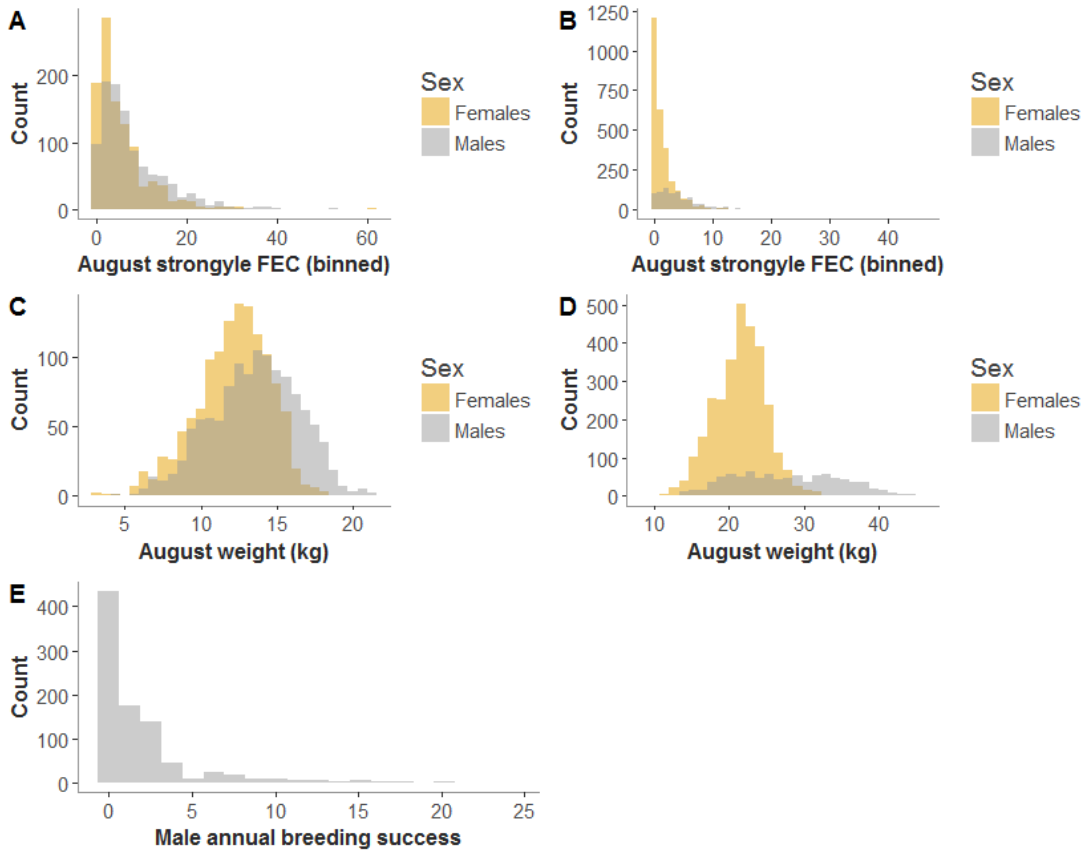

Figure 3.1. Histograms of August strongyle FEC in lambs (A) and adults (B), August weight in lambs (C) and adults (C) and annual breeding success in adult male Soay sheep (E).

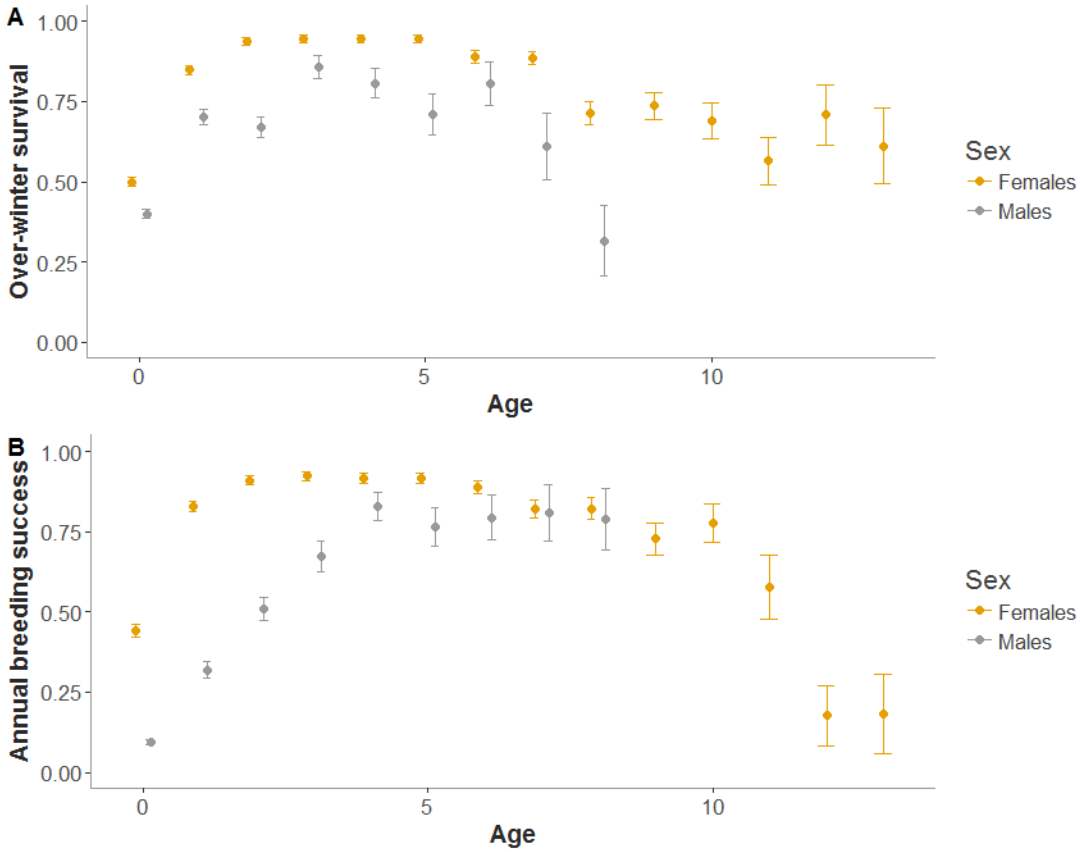

Figure 3.2. Mean over-winter survival (A) and annual breeding success (B) probabilities by age and sex in Soay sheep. Female sheep older than 13 years (n = 6) were included in the 13 year age group, while male sheep older than 8 years (n = 5) were included in the 8 year age group. SE bars are displayed.

3.4 Results

3.4.1 Associations between antibody levels and strongyle faecal egg count

In lambs, August strongyle FEC was negatively associated with all three anti-Tc antibody isotype (IgA, IgG and IgE) levels (Table 3.1, Figure 3.3). For all three isotypes, August FEC decreased linearly with increasing antibody levels. In a model containing all three antibody isotypes, IgA levels were independently negatively associated with FEC (b = -0.074±0.021 SE, $\chi^2_{(1)} = 11.900$, $p < 0.001$), while IgE and IgG were no longer significantly associated with FEC (IgE: b = -0.031±0.020 SE, $\chi^2_{(1)} = 2.260$, p = 0.133; IgG: b = -0.035±0.022 SE, $\chi^2_{(1)} = 2.600$, p = 0.107). We found no evidence for sex or density-dependent effects of antibody levels on FEC in lambs (Table 3.1).

In adults, there was a negative linear association between anti-Tc IgG levels and August FEC for both males and females (Table 3.2, Figure 3.3). In females, there was also a weak curvilinear relationship of IgA with FEC (Table 3.2, Figure 3.3). A model with both significant antibody terms for females showed these two associations to be independent (IgG: b = -0.116±0.029 SE, $\chi^2_{(1)} = 15.72$, $p < 0.001$; IgA: b(IgA^2)= 0.312±0.120 SE, b(IgA) = -0.338±0.119 SE, $\chi^2_{(1)} = 6.72$, p = 0.010). Model predictions suggested that FEC was lowest at intermediate anti-Tc IgA levels in adult females (Figure 3.2), and without the quadratic term in the model there was not a significant linear association between anti-Tc IgA and FEC (b = -0.049±0.029 SE, $\chi^2_{(1)} = 2.920$, p = 0.087). We found no evidence for density-dependent effects of antibody levels on FEC in adults (Table 3.2).

Table 3.1. GLMM results of the final minimal model for August strongyle FEC for lambs (both sexes). Included are the estimated effects (estimate), standard error (SE) and the significance of fixed effects based on a likelihood ratio test (LRT, d.f., p-value). Dropped fixed effects show the significance of adding dropped terms back to the minimal model. For the added fixed effects, each antibody measure was added separately to the minimal model, including their linear and quadratic terms. Where the quadratic terms were significant, estimated effects are stated for both the linear and quadratic terms combined, and interactions were tested in addition to both terms.

	August FEC - lambs Obs/IDs: n=1865				
variables	estimate	SE	LRT	d.f.	p-value
fixed effects					
intercept	1.555	0.069			
sex (male)	0.423	0.039	114.200	1	<0.001
twin	0.179	0.048	13.840	1	<0.001
weight	-0.921	0.127	-	-	-
weight^2	0.707	0.129	30.900	1	<0.001
population size	0.236	0.064	11.100	1	<0.001
dropped fixed effects					
age			0.480	1	0.488
age^2			1.280	2	0.527
added fixed effects					
IgA	**-0.096**	**0.019**	**25.600**	**1**	**<0.001**
IgA^2			0.580	1	0.446
IgE	**-0.059**	**0.019**	**9.140**	**1**	**0.003**
IgE^2			1.100	1	0.294
IgG	**-0.074**	**0.019**	**14.820**	**1**	**<0.001**
IgG^2			1.980	1	0.159
sex*IgA			0.080	1	0.777
sex*IgE			0.620	1	0.431
sex*IgG			0.040	1	0.842
population*IgA			2.660	1	0.103
population*IgE			1.720	1	0.190
population*IgG			0.040	1	0.842

Table 3.2. GLMM results of the final minimal model for August strongyle FEC for adult females and adult males. Included are the estimated effects (estimate), standard error (SE) and the significance of fixed effects based on a likelihood ratio test (LRT, d.f., p-value). Dropped fixed effects show the significance of adding dropped terms back to the minimal model. For the added fixed effects, each antibody measure was added separately to the minimal model, including their linear and quadratic terms. Where the quadratic terms were significant, estimated effects are stated for both the linear and quadratic terms combined, and interactions were tested in addition to both terms.

		August FEC - adults									
		Females	Obs: n= 2715		IDs: n= 875		**Males**	Obs: n= 848		IDs: n= 452	
	variables	estimate	SE	LRT	d.f.	p-value	estimate	SE	LRT	d.f.	p-value
fixed effects	intercept	0.092	0.053				1.315	0.068			
	age	-0.636	0.111	-	-	-	0.496	0.170	-	-	-
	age^2	0.603	0.102	35.000	1	<0.001	-0.318	0.135	5.500	1	0.019
	weight	-0.401	0.039	102.680	1	<0.001	-1.000	0.217	-	-	-
	$weight^2$	-	-	-	-	-	0.643	0.226	8.020	1	0.005
	population size	-0.037	0.048	0.580	1	0.4463	0.054	0.063	0.740	1	0.390
dropped fixed effects	$weight^2$			0.920	1	0.3375					
added fixed effects	IgA	**-0.337**	**0.120**	-	-	-			0.460	1	0.498
	IgA^2	**0.299**	**0.121**	**6.140**	**1**	**0.013**			0.040	1	0.842
	IgE			0.220	1	0.639			1.480	1	0.224
	IgE^2			0.820	1	0.365			0.220	1	0.639
	IgG	**-0.120**	**0.029**	**16.980**	**1**	**<0.001**	**-0.093**	**0.032**	**8.420**	**1**	**0.004**
	IgG^2			0.080	1	0.777			0.300	1	0.584
	population*IgA			1.880	1	0.170			3.620	1	0.057
	population*IgE			0.060	1	0.807			2.720	1	0.099
	population*IgG			3.580	1	0.058			0.520	1	0.471

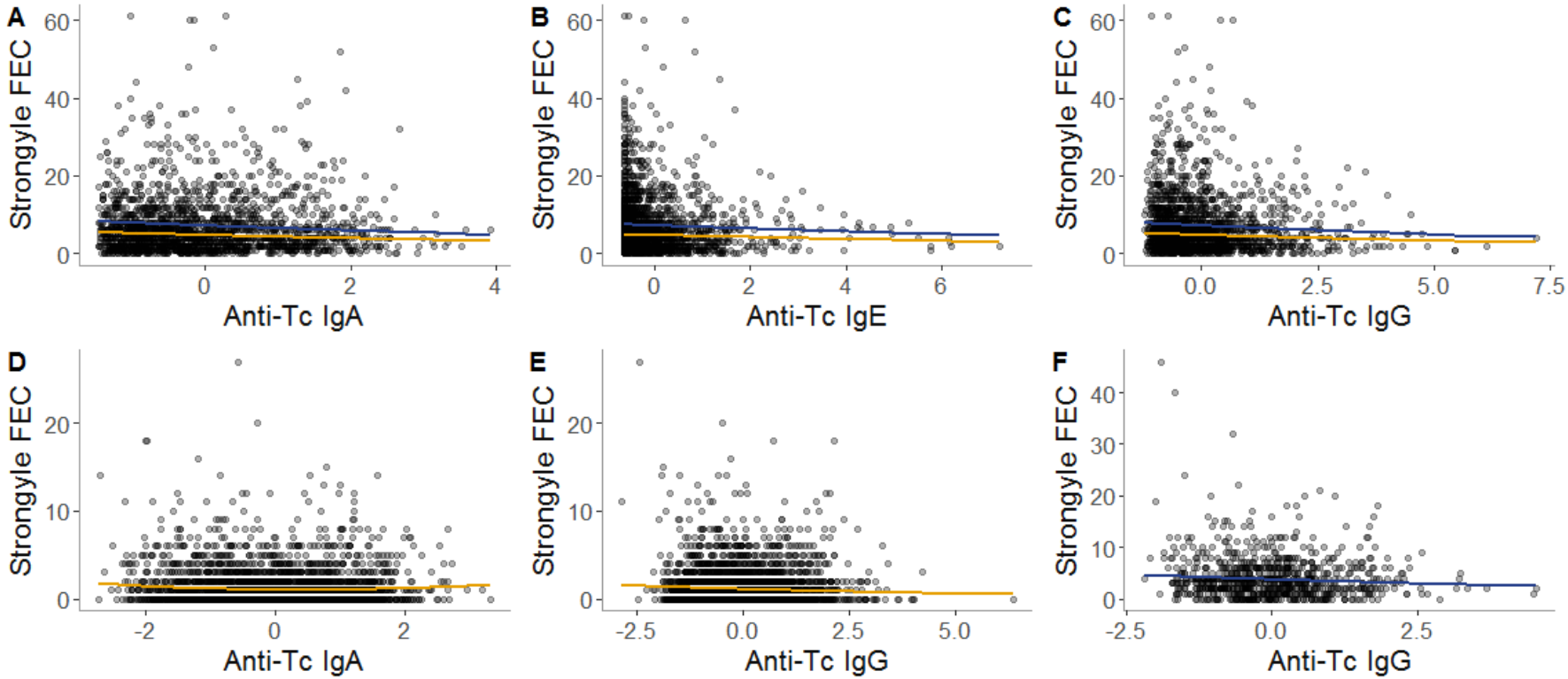

Figure 3.3. Scatterplots of raw data and GLMM predictions for associations between anti-*T. circumcincta* IgA, IgE and IgG levels and strongyle FEC (binned, see Methods). Predictions are shown as blue lines for males and yellow lines for females, and are estimated at average values for all continuous fixed effects in the minimal model, and singletons for lambs. Associations are shown between anti-Tc IgA, IgE and IgG and strongyle FEC in lambs (A-C), anti-Tc IgA and IgG for female adults (D-E) and anti-Tc IgG for adult males (F).

3.4.2 Associations between antibody levels and weight

There was a weak quadratic association between August weight and anti-Tc IgE levels in lambs (Table 3.3). Model predictions suggested that weight increased and then plateaued with increasing IgE levels in lambs (Figure 3.4). Without the quadratic in the model there was a significant positive linear association between IgE and weight (b = 0.128±0.042 SE, $\chi^2_{(1)} = 9.399$, p = 0.002). This association was not driven by a few individuals with high anti-Tc IgE levels, as a quadratic association remained after removing the 40 individuals with scaled IgE levels > 3 ($b(IgE^2) = -1.019\pm0.294$ SE, b(IgE) = 0.704±0.154 SE, $\chi^2_{(1)} = 11.996$, p = 0.001). We found no association of the other antibody isotypes and weight in lambs, or antibody by sex or density interactions (Table 3.3).

In adult females, there was positive linear association between August weight and anti-Tc IgE levels and a curvilinear association was found between IgA and IgG levels and weight (Table 3.4, Figure 3.4). While higher IgE levels were associated with heaviest August weights, it was the intermediate anti-Tc IgA and IgG levels that were associated with heaviest weights (Figure 3.4). These associations were not driven by a few individuals with high anti-Tc antibody levels, as the association remained after removal of individuals with high antibody levels of each isotype (after removal of IgA levels >2.5 (n=15), $\chi^2_{(1)} = 10.235$, p = 0.001; after removal of IgE levels >2.5 (n=61), $\chi^2_{(1)} = 6.981$, p = 0.008; after removal of IgG levels >3.75 (n=5), $\chi^2_{(1)} = 26.621$, p<0.001). Linear associations between weight and IgA and IgG were considerably weaker and less significant than quadratic effects (IgA: b = 0.106±0.051 SE, $\chi^2_{(1)} = 4.202$, p = 0.040; IgG: b = 0.085±0.044 SE, $\chi^2_{(1)} = 3.683$, p = 0.055). When all the significant antibody terms were in the same model, all remained significant, proving each antibody isotype had independent associations with August weight (IgA: $b(IgA^2) = -0.621\pm0.187$ SE, b(IgA) = 0.667± 0.189 SE, $\chi^2_{(1)} = 11.075$, p < 0.001; IgE: b = 0.156±0.053 SE, $\chi^2_{(1)} = 8.934$, p = 0.003; IgG: $b(IgG^2) = -0.605\pm0.145$ SE, b(IgG) = 0.674±0.154 SE, $\chi^2_{(1)} = 17.413$, p < 0.001). A marginal density-dependent association with IgA was found, indicating a slightly stronger increase with weight at high sheep densities (Table 3.4). The interaction remained significant when the other antibody terms were included in the model (b = 0.077±0.031 SE, $\chi^2_{(1)} = 6.262$, p = 0.012). In adult males we found a positive linear association of IgA with weight, but no association with the other antibody isotypes or density interactions (Table 3.4, Figure 3.4).

Table 3.3. LMM results of the final minimal model for August weight for lambs (both sexes). Included are the estimated effects (estimate), standard error (SE) and the significance of fixed effects based on a likelihood ratio test (LRT, d.f., p-value). For the added fixed effects, each antibody measure was added separately to the minimal model, including their linear and quadratic terms. Where the quadratic terms were significant, estimated effects are stated for both the linear and quadratic terms combined, and interactions were tested in addition to both terms.

	August weight - lambs				
	Both sexes			Obs/IDs=1992	
variables	estimate	SE	LRT	d.f.	p-value
fixed effects					
intercept	12.869	0.221			
sex (males)	1.448	0.079	306.950	1	<0.001
twin	-3.299	0.112	711.190	1	<0.001
age (days)	0.383	0.051	54.670	1	<0.001
maternal age (years)	4.236	0.166	-	-	-
maternal age^2	-3.936	0.166	482.220	1	<0.001
population size	-0.409	0.201	4.153	1	0.042
added fixed effects					
IgA			0.306	1	0.580
IgA^2			0.161	1	0.688
IgE	**0.281**	**0.086**	-	-	-
IgE^2	**-0.169**	**0.083**	**4.191**	**1**	**0.041**
IgG			0.531	1	0.466
IgG^2			3.155	1	0.076
sex*IgA			0.036	1	0.851
sex*IgE			0.794	1	0.373
sex*IgG			1.788	1	0.181
population*IgA			0.023	1	0.881
population*IgE			0.152	1	0.697
population*IgG			1.100	1	0.294

Table 3.4. LMM results of the final minimal model for August weight for adult females and adult males. Included are the estimated effects (estimate), standard error (SE) and the significance of fixed effects based on a likelihood ratio test (LRT, d.f., p-value). Dropped fixed effects show the significance of adding dropped terms back to the minimal model. For the added fixed effects, each antibody measure was added separately to the minimal model, including their linear and quadratic terms. Where the quadratic terms were significant, estimated effects are stated for both the linear and quadratic terms combined, and interactions were tested in addition to both terms.

	August weight - adults									
	Females	Obs: n= 3000		IDs: n= 918		**Males**	Obs: n= 931		IDs: n= 482	
variables	estimate	SE	LRT	d.f.	p-value	estimate	SE	LRT	d.f.	p-value
fixed effects										
intercept	21.296	0.130				27.178	0.232			
age	5.835	0.106	-	-	-	14.190	0.322	-	-	-
age^2	-4.331	0.105	1276.400	1	<0.001	-9.415	0.310	575.120	1	<0.001
population size	-0.548	0.111	18.927	1	<0.001	-0.450	0.187	5.667	1	0.017
added fixed effects										
IgA	**0.759**	**0.188**	-	-	-	**0.361**	**0.133**	**7.289**	**1**	**0.007**
IgA^2	**-0.673**	**0.187**	**13.069**	**1**	**<0.001**			2.668	1	0.102
IgE	**0.186**	**0.052**	**12.889**	**1**	**<0.001**			1.697	1	0.193
IgE^2			0.004	1	0.947			0.002	1	0.966
IgG	**0.753**	**0.153**	-	-	-			0.241	1	0.623
IgG^2	**-0.659**	**0.144**	**20.734**	**1**	**<0.001**			2.027	1	0.155
population*IgA	**0.075**	**0.031**	**6.109**	**1**	**0.013**			0.201	1	0.654
population*IgE			0.715	1	0.398			3.093	1	0.079
population*IgG			0.786	1	0.375			0.081	1	0.776

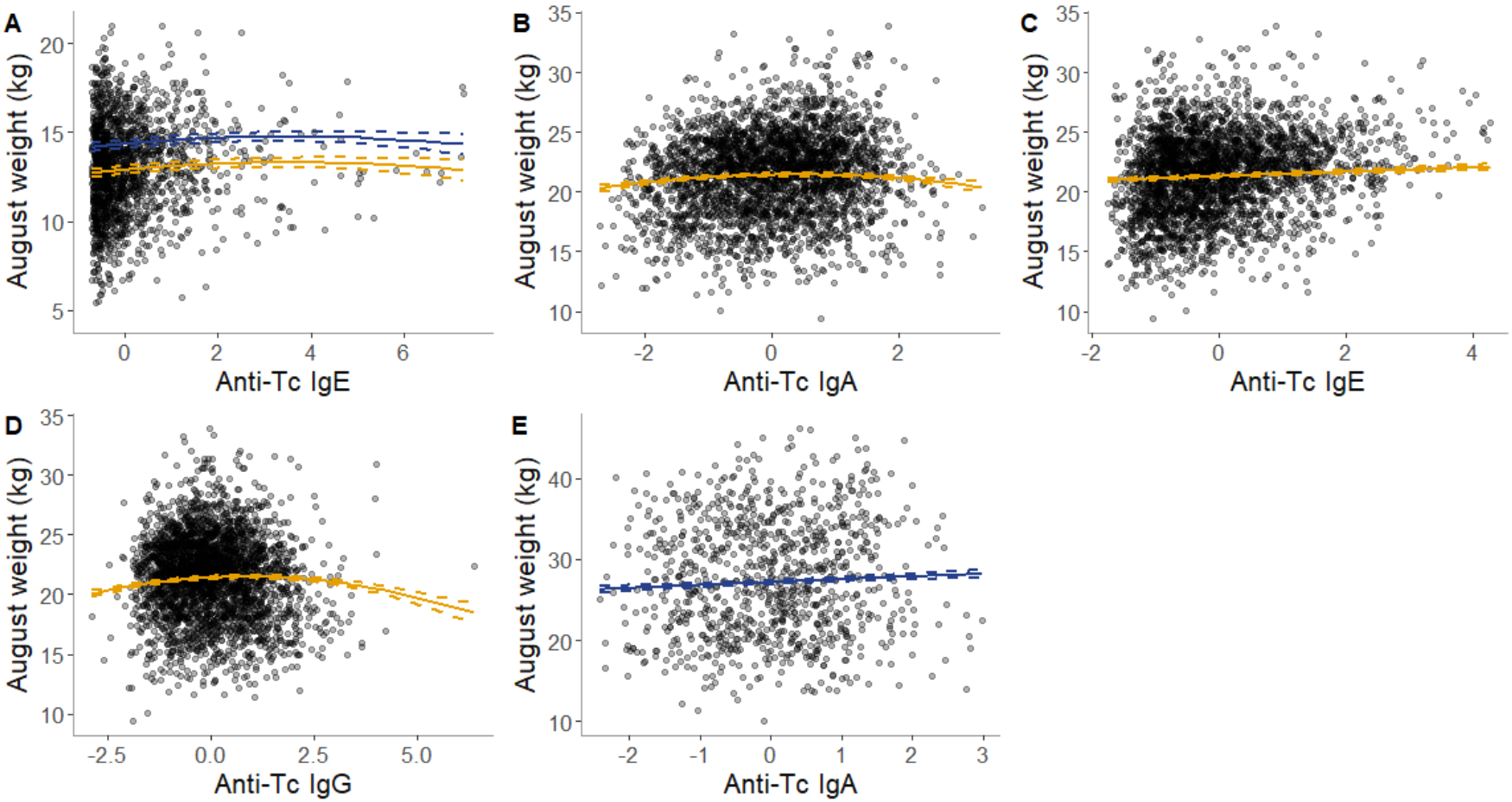

Figure 3.4. Scatterplots of raw data and LMM predictions for associations between anti-*T. circumcincta* IgA, IgE and IgG levels and weight. Predictions are shown as blue lines for males and yellow lines for females, and are estimated at average values for all continuous fixed effects in the minimal model, and singletons for lambs. Associations are shown between anti-Tc IgE and weight in lambs (A), anti-Tc IgA and weight for adult males (B) and anti-Tc IgA, IgE and IgG for adult females (F).

3.4.3 Associations between antibody levels and over-winter survival

We found little evidence for associations between any of the three anti-Tc antibody levels and survival in lambs or adult males (Table 3.5-3.6). Although there were no significant interactions between antibody levels and sex for lambs, we found a marginally significant interaction between anti-Tc IgA levels and population density. This predicted a weakening of selection at high density despite the overall effect of IgA in these models being marginally non-significant and positive (Table 3.5). We found anti-Tc IgG and IgE levels were significant predictors of over-winter survival in adult females (Table 3.6, Figure 3.5). Females with higher IgG and IgE levels were more likely to survive the winter, independent of age and weight (Table 3.6, Figure 3.5). In a model with both IgG and IgE fitted, IgE no longer predicted over-winter survival, but IgG remained significant (IgE: b = 0.127±0.071 SE, $\chi^2_{(1)} = 3.202$, p = 0.074; IgG: b = 0.195±0.072 SE, $\chi^2_{(1)} = 7.357$, p = 0.007). In addition, IgG was significant if strongyle FEC was included in the model (b = 0.243±0.078 SE, $\chi^2_{(1)} = 9.907$, p = 0.002) and if the one individual with high IgG was dropped (b = 0.235±0.085 SE, $\chi^2_{(1)} = 8.240$, p = 0.004). There was no evidence for environment-dependent selection on adult females.

Table 3.5. GLMM results of the final minimal model for over-winter survival for lambs (both sexes). Included are the estimated effects (estimate), standard error (SE) and the significance of fixed effects based on a likelihood ratio test (LRT, d.f., p-value). Dropped fixed effects show the significance of adding dropped terms back to the minimal model. For the added fixed effects, each antibody measure was added separately to the minimal model, including their linear and quadratic terms. Where the quadratic terms were significant, effects are stated for both the linear and quadratic terms combined, and interactions were tested in addition to both terms.

	Over-winter survival - lambs n=2241				
variables	estimate	SE	LRT	d.f.	p-value
fixed effects					
intercept	0.170	0.244			
sex (male)	-1.006	0.119	-	-	-
weight	0.986	0.099	-	-	-
population size	-1.252	0.232	20.119	1	<0.001
sex (male)*weight	-0.331	0.127	6.812	1	0.009
dropped fixed effects					
twin			0.776	1	0.378
weight^2			0.959	1	0.328
sex*weight^2			2.553	2	0.279
added fixed effects					
IgA			3.102	1	0.078
IgA^2			0.008	1	0.928
IgE			0.348	1	0.555
IgE^2			0.192	1	0.661
IgG			0.043	1	0.837
IgG^2			<0.001	1	0.988
sex*IgA			1.241	1	0.265
sex*IgE			0.021	1	0.884
sex*IgG			0.234	1	0.629
population*IgA	**-0.130**	**0.066**	**3.882**	**1**	**0.049**
population*IgE			0.492	1	0.483
population*IgG			0.679	1	0.410

Table 3.6. GLMM results of the final minimal model for over-winter survival for adult females and males. Included are the estimated effects (estimate), standard error (SE) and the significance of fixed effects based on a likelihood ratio test (LRT, d.f., p-value). Dropped fixed effects show the significance of adding dropped terms back to the minimal model. For the added fixed effects, each antibody measure was added separately to the minimal model, including their linear and quadratic terms. Where the quadratic terms were significant, effects are stated for both the linear and quadratic terms combined, and interactions were tested in addition to both terms.

	Over-winter survival - adults									
	Females	Obs: n= 2960		IDs: n= 918		**Males**	Obs: n= 860		IDs: n= 443	
variables	estimate	SE	LRT	d.f.	p-value	estimate	SE	LRT	d.f.	p-value
fixed effects										
intercept	2.916	0.239				1.551	0.336			
age	-1.242	0.083	259.600	1	<0.001	-1.029	0.166	41.892	1	<0.001
weight	2.440	0.560	-	-	-	1.169	0.172	52.879	1	<0.001
weight^2	-1.268	0.583	4.430	1	0.035					
population size	-0.725	0.233	8.562	1	0.003	-0.908	0.326	7.620	1	0.006
dropped fixed effects										
age^2			<0.001	1	0.986			0.5818	1	0.4456
weight^2								0.4568	1	0.4991
added fixed effects										
IgA			0.524	1	0.469			2.484	1	0.115
IgA^2			0.043	1	0.837			0.017	1	0.898
IgE	**0.157**	**0.071**	**5.013**	**1**	**0.025**			0.007	1	0.936
IgE^2			0.770	1	0.380			0.165	1	0.685
IgG	**0.213**	**0.072**	**9.004**	**1**	**0.003**			<0.001	1	0.996
IgG^2			1.590	1	0.207			0.181	1	0.670
population*IgA			0.001	1	0.972			0.146	1	0.702
population*IgE			<0.001	1	0.994			1.196	1	0.274
population*IgG			2.483	1	0.115			0.150	1	0.698

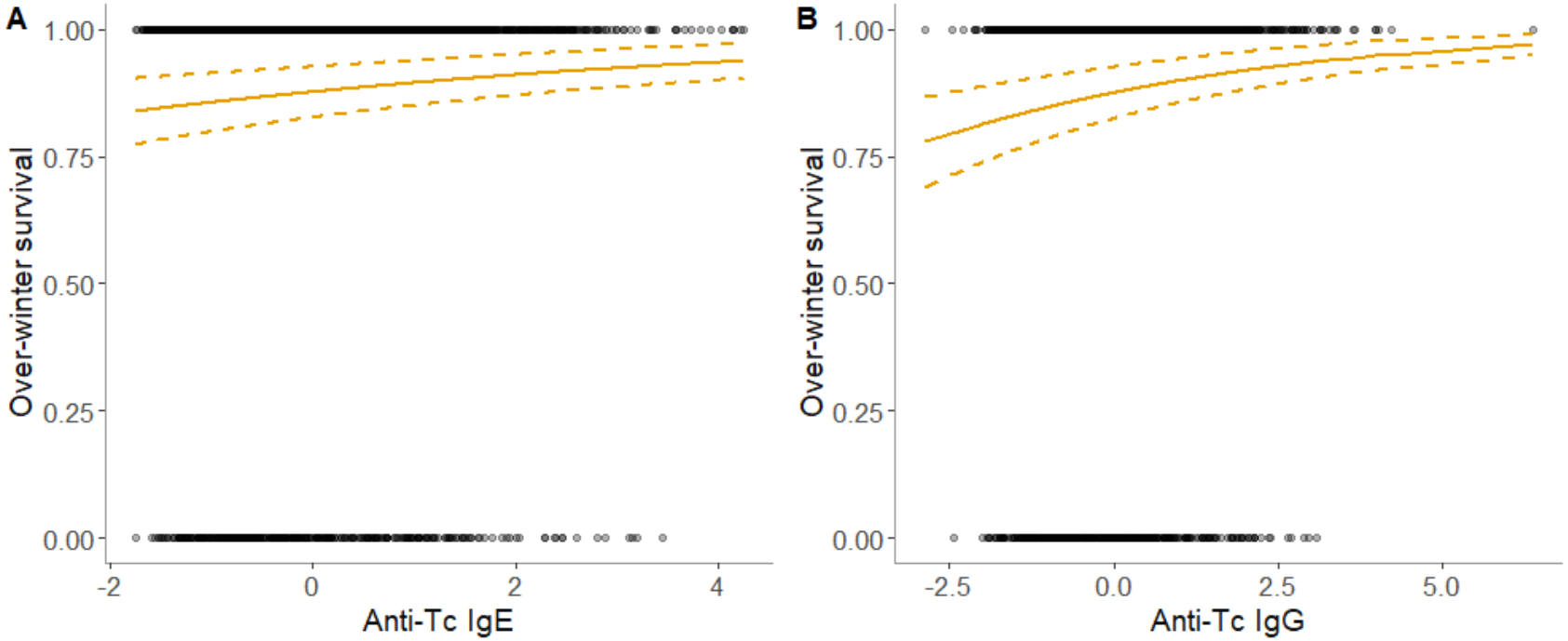

Figure 3.5. Scatterplots of raw data and GLMM predictions for associations between anti-*T. circumcincta* IgE (A) and IgG (B) levels and subsequent over-winter survival for adult female Soay sheep. Prediction lines are estimated for adult females of average weight and age at high Village Bay population size (n=672).

3.4.4 Associations between antibody levels and annual breeding success

In female lambs, we found a negative association between anti-Tc IgE levels and breeding success the next year (Table 3.7). Although our initial model detected a significant quadratic association between IgE and female lamb breeding success (Table 3.7), visual inspection of this relationship and the model predictions suggested it could be driven by a handful of lambs with very high antibody levels . When the 4 individuals with anti-Tc IgE values > 4 were dropped, the quadratic was no longer significant ($\chi^2_{(1)} = 0.919$, p = 0.338), but there was a significant negative linear relationship between IgE levels and lamb breeding success (b = -0.298±0.118 SE, $\chi^2_{(1)} = 6.661$, p = 0.010; Figure 3.6A). We found no associations with any other antibody isotype, or density interactions in female lambs. In male lambs, we found a positive association between IgE and breeding success the next year (Table 3.9). Male lambs with higher IgE antibody levels were more likely to sire offspring in the subsequent year (Figure 3.6B) and this association remained after dropping the six individuals with high IgE levels (b = 0.353±0.113 SE, $\chi^2_{(1)} = 9.037$, p = 0.003). We found no associations with any other antibody isotype or environment dependent interactions.

We found no evidence for associations between anti-Tc IgA, IgE and their interactions with density on annual breeding success in female adults (Table 3.8). While testing associations between IgA and their interactions with density, we suffered from problems with model convergence with weight as a quadratic term in the model. When weight was not included in the model, IgA had no association with annual breeding success. We found a curvilinear association of anti-Tc IgG on breeding success, but this result was extremely weak and marginally significant (Table 3.8). There was no underlying linear association between IgG and annual breeding success (b = -0.014±0.132 SE, $\chi^2_{(1)} = 0.011$, p = 0.918). There was no association between antibody levels and female annual reproductive success, nor any evidence for interactions between antibody levels and density in any model of female reproduction (Table 3.8). There was a significant positive association between IgG levels and the breeding success of adult males the following spring (Table 3.9). Males with higher IgG levels in August sired more lambs in the subsequent year independent of age and body weight (Figure 3.6C). We found no associations with any other antibody isotypes or density interactions in adult males.

Table 3.7. GLMM results of the final minimal model for annual breeding success in female lambs. Included are the estimated effects (estimate), standard error (SE) and the significance of fixed effects based on a likelihood ratio test (LRT, d.f., p-value). Dropped fixed effects show the significance of adding dropped terms back to the minimal model. For the added fixed effects, each antibody measure was added separately to the minimal model, including their linear and quadratic terms. Where the quadratic terms were significant, estimated effects are stated for both the linear and quadratic terms combined, and interactions were tested in addition to both terms.

Annual breeding success - female lambs n=595					
variables	estimate	SE	LRT	d.f.	p-value
fixed effects					
intercept	-0.441	0.201			
weight	0.614	0.109	35.353	1	<0.001
population size	-0.657	0.196	9.241	1	0.002
dropped fixed effects					
twin			0.034	1	0.853
weight^2			2.335	1	0.127
added fixed effects					
IgA			0.740	1	0.390
IgA^2			0.452	1	0.502
IgE	**-0.650**	**0.230**			
IgE^2	**0.564**	**0.254**	**6.273**	**1**	**0.012**
IgG			0.078	1	0.780
IgG^2			0.532	1	0.466
population*IgA			0.115	1	0.735
population*IgE			1.561	1	0.212
population*IgG			0.007	1	0.936

Table 3.8. GLMM results of the final minimal model for annual breeding and reproductive success in female adults. Included are the estimated effects (estimate), standard error (SE) and the significance of fixed effects based on a likelihood ratio test (LRT, d.f., p-value). Dropped fixed effects show the significance of adding dropped terms back to the minimal model. For the added fixed effects, each antibody measure was added separately to the minimal model, including their linear and quadratic terms. Where the quadratic terms were significant, estimated effects are stated for both the linear and quadratic terms combined, and interactions were tested in addition to both terms. $^{+}$ - indicates problems with model convergence, results are quoted from a model without weight terms in the model.

	Annual breeding success - female adults					**Annual reproductive success - female adults**				
	Obs: n= 2603		IDs: n= 792			Obs: n= 2255		IDs: n= 732		
variables	estimate	SE	LRT	d.f.	p-value	estimate	SE	LRT	d.f.	p-value
fixed effects										
intercept	6.363	0.638				2.338	0.168			
age	1.932	0.483	-	-	-	1.715	0.303	-	-	-
age^2	-2.414	0.435	34.527	1	<0.001	-1.780	0.264	45.128	1	<0.001
weight	2.188	1.051	-	-	-	3.747	0.629	-	-	-
weight^2	-2.245	1.046	4.409	1	0.036	-3.377	0.619	27.651	1	<0.001
population size	-0.153	0.125	1.425	1	0.233	-0.433	0.144	8.280	1	0.004
added fixed effects										
IgA			0.079^{+}	1^{+}	0.7791^{+}			2.757	1	0.097
IgA^2			3.798^{+}	1^{+}	0.051^{+}			0.100	1	0.752
IgE			0.869	1	0.351			0.104	1	0.748
IgE^2			0.735	1	0.391			1.464	1	0.226
IgG	**-1.031**	**0.557**						0.108	1	0.742
IgG^2	**0.998**	**0.532**	**3.913**	**1**	**0.048**			0.011	1	0.918
population*IgA			0.887	1	0.346			0.024	1	0.876
population*IgE			0.594	1	0.4409			0.139	1	0.710
population*IgG			0.566	1	0.452			2.325	1	0.127

Table 3.9. GLMM results of the final minimal model for annual breeding success in male lambs and adults. Included are the estimated effects (estimate), standard error (SE) and the significance of fixed effects based on a likelihood ratio test (LRT, d.f., p-value). Dropped fixed effects show the significance of adding dropped terms back to the minimal model. For the added fixed effects, each antibody measure was added separately to the minimal model, including their linear and quadratic terms. Where quadratic terms were significant, effects are stated for both the linear and quadratic terms combined, and interactions were tested in addition to both terms.

Annual breeding success - male lambs						**Annual breeding success - male adults**					
		n= 1118					Obs: n= 891		IDs: n= 459		
variables	estimate	SE	LRT	d.f.	p-value	variables	estimate	SE	LRT	d.f.	p-value
fixed effects						***fixed effects***					
intercept	-3.101	0.212				intercept	-0.898	0.213			
weight	4.566	1.563	-	-	-	age	0.199	0.060	10.780	1	0.001
weight^2	-3.568	1.402	8.063	1	0.005	weight	0.812	0.074	112.720	1	<0.001
population size	-0.918	0.143	26.969	1	<0.001	horn type (normal)	0.758	0.193	14.880	1	<0.001
						population size	-0.251	0.103	5.240	1	0.022
dropped fixed effects						***dropped fixed effects***					
horn type			0.034	1	0.855	weight^2			0.220	1	0.639
twin			0.786	1	0.375	age^2			2.800	1	0.094
added fixed effects						***added fixed effects***					
IgA			2.763	1	0.096	IgA			3.220	1	0.073
IgA^2			0.166	1	0.684	IgA^2			0.000	1	1.000
IgE	**0.263**	**0.090**	**7.753**	**1**	**0.005**	IgE			1.140	1	0.286
IgE^2			1.478	1	0.224	IgE^2			0.020	1	0.888
IgG			0.967	1	0.326	IgG	**0.106**	**0.048**	**4.780**	**1**	**0.029**
IgG^2			0.327	1	0.567	IgG^2			0.980	1	0.322
population*IgA			0.110	1	0.740	population*IgA			0.260	1	0.610
population*IgE			0.059	1	0.809	population*IgE			1.580	1	0.209
population*IgG			0.705	1	0.401	population*IgG			2.760	1	0.097

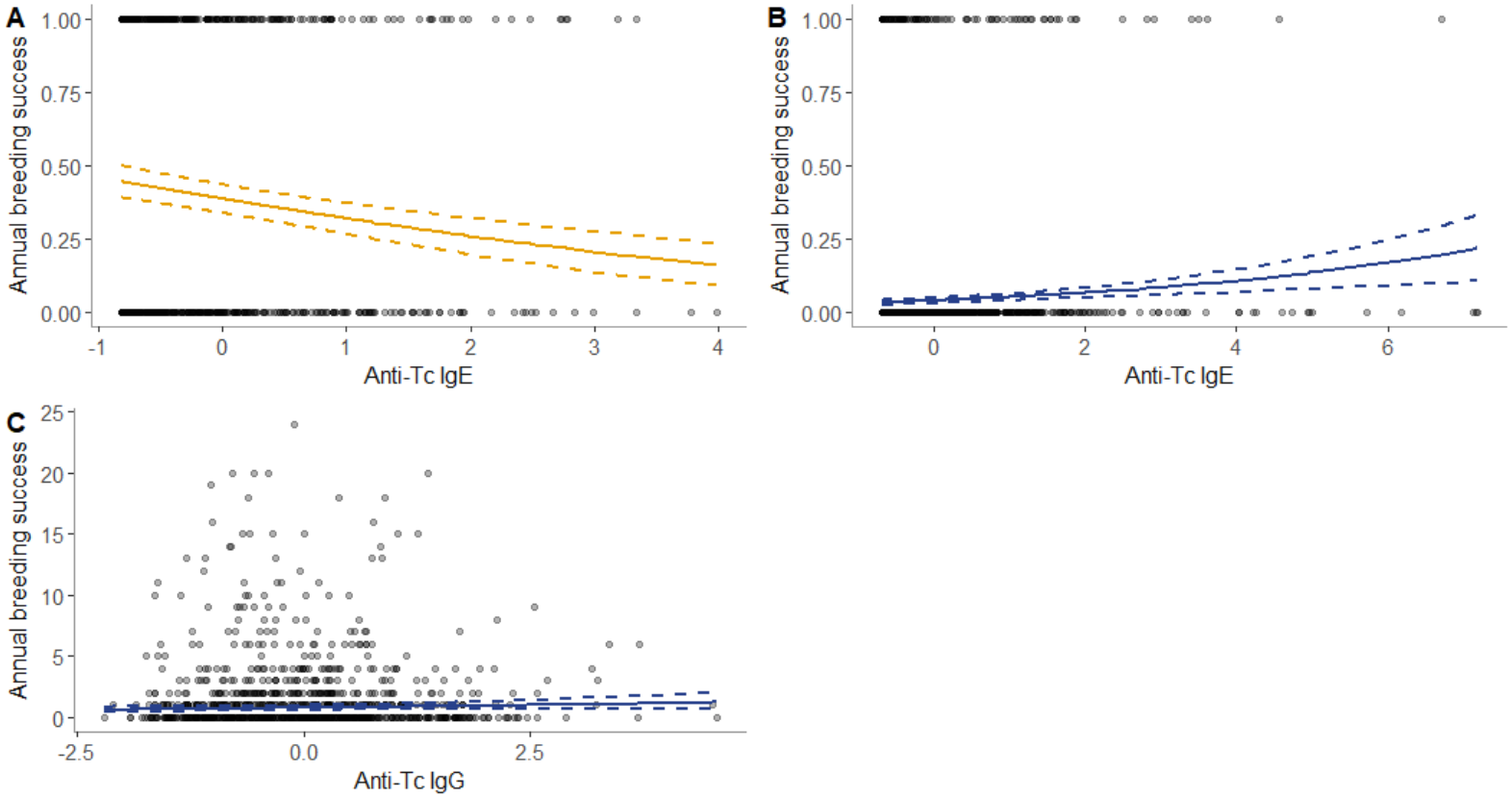

Figure 3.6. Scatterplots of raw data and GLMM predictions for associations between anti-*T. circumcincta* IgE and IgG levels and subsequent breeding success in lambs and adult males. Associations are shown between anti-Tc IgE and the probability of female sheep breeding in their first year with 4 IgE outliers removed (A), between anti-Tc IgE and the probability of male sheep breeding in their first year (B) and between anti-Tc IgG and annual breeding success for adult males (C). Prediction lines are estimated at average values for all continuous fixed effects in the minimal model, with dashed lines indicating the standard error of these estimates.

3.4.5 Associations between SNPs and fitness

We looked at the associations between 13 SNPs that were found to be associated with anti-Tc IgA levels (Chapter 2) and over-winter survival and annual breeding success. No SNPs passed the Bonferroni threshold of $p < 0.004$ for over-winter survival or annual breeding success in lambs or adults in either sex (Table 3.10-3.11).

Table 3.10. GLMM results of the association between SNPs associated with anti-Tc IgA and subsequent over-winter survival. Included are the estimated effects (estimate), standard error (SE) and the significance of fixed effects based on a likelihood ratio test (LRT, d.f., p-value). Added fixed effects show the significance of adding SNPs separately back to the minimal model (detailed in Table 3.3).

	Over-winter survival - lambs						**Over-winter survival - adults**					
	Both sexes						**Females**			**Males**		
variables	estimate	SE	LRT	d.f.	p-value	variables	LRT	d.f.	p-value	LRT	d.f.	p-value
added fixed effects						***added fixed effects***						
s57548.1			2.307	2	0.316	s57548.1	1.294	2	0.524	3.370	2	0.185
OAR24_8358348.1			2.592	2	0.274	OAR24_8358348.1	0.161	2	0.923	0.295	2	0.863
s35996.1			2.661	2	0.264	s35996.1	1.662	2	0.436	3.277	2	0.194
s33564.1			2.652	2	0.266	s33564.1	1.621	2	0.445	3.277	2	0.194
OAR24_10569180.1			2.962	2	0.227	OAR24_10569180.1	1.449	2	0.485	1.169	2	0.557
OAR24_11975271.1			3.881	2	0.144	OAR24_11975271.1	1.523	2	0.467	2.260	2	0.323
OAR24_12006191.1			1.846	2	0.397	OAR24_12006191.1	3.777	2	0.151	0.987	2	0.611
s29806.1 (A/A)	0.000	0.000	**8.336**	**2**	**0.015**	s29806.1	0.502	2	0.778	3.893	2	0.143
s29806.1 (A/G)	-0.111	0.120				s11845.1	3.817	2	0.148	0.252	2	0.882
s29806.1 (G/G)	-0.731	0.260				s06827.1	1.784	2	0.410	0.114	2	0.945
s11845.1 (A/A)	0.000	0.000	**6.290**	**2**	**0.043**	s71593.1	0.807	2	0.668	0.139	2	0.933
s11845.1 (A/G)	0.047	0.122				s61066.1	1.269	2	0.530	2.729	2	0.256
s11845.1 (G/G)	-0.351	0.167				OAR24_13675640.1	1.119	2	0.572	5.601	2	0.061
s06827.1			1.212	2	0.546							
s71593.1			4.204	2	0.122							
s61066.1 (A/A)	0.000	0.000	**7.845**	**2**	**0.020**							
s61066.1 (A/G)	-0.166	0.117										
s61066.1 (G/G)	-0.626	0.236										
OAR24_13675640.1			3.029	2	0.220							

Table 3.11. GLMM results of the association between SNPs associated with anti-Tc IgA and annual breeding success. Included are the estimated effects (estimate), standard error (SE) and the significance of fixed effects based on a likelihood ratio test (LRT, d.f., p-value). Added fixed effects show the significance of adding SNPs separately back to the minimal model (detailed in Table 3.4-3.6).

	Annual breeding success - females						**Annual breeding success - males**					
	Lambs			Adults			Lambs			Adults		
variables	LRT	d.f.	p-value	LRT	d.f.	p-value	LRT	d.f.	p-value	LRT	d.f.	p-value
added fixed effects												
s57548.1	0.153	2	0.926	1.542	2	0.463	0.322	2	0.851	5.560	2	0.062
OAR24_8358348.1	4.827	2	0.090	0.740	2	0.691	0.679	2	0.712	2.380	2	0.304
s35996.1	0.442	2	0.802	1.506	2	0.471	0.302	2	0.860	5.760	2	0.056
s33564.1	0.481	2	0.786	1.521	2	0.468	0.332	2	0.847	5.760	2	0.056
OAR24_10569180.1	0.576	2	0.750	1.947	2	0.378	0.714	2	0.700	3.740	2	0.154
OAR24_11975271.1	3.111	2	0.211	0.198	2	0.906	2.595	2	0.273	0.220	2	0.896
OAR24_12006191.1	2.536	2	0.281	2.524	2	0.283	1.860	2	0.395	0.460	2	0.795
s29806.1	0.210	2	0.901	0.215	2	0.898	1.438	2	0.487	0.880	2	0.644
s11845.1	0.394	2	0.821	1.839	2	0.399	2.857	2	0.240	0.520	2	0.771
s06827.1	1.437	2	0.487	0.589	2	0.745	1.245	2	0.537	1.780	2	0.411
s71593.1	0.664	2	0.718	0.063	2	0.969	2.533	2	0.282	0.740	2	0.691
s61066.1	3.028	2	0.220	0.460	2	0.794	0.541	2	0.763	0.460	2	0.795
OAR24_13675640.1	1.326	2	0.515	2.016	2	0.365	0.801	2	0.670	0.100	2	0.951

3.5 Discussion

In this study, we looked for associations between helminth-specific immune responses and health and fitness consequences. Theory predicts that stabilising selection is most likely to be occurring on immune traits, due to energetic costs and immunopathological consequences, but few studies have empirically tested for this (Viney *et al.* 2005; Graham *et al.* 2005; Seppälä 2015). Here, we looked for both linear and quadratic forms of selection on antibodies and checked for age, sex and environment-specific associations with fitness. We found complex associations with fitness that were not consistent across antibody isotypes. This is supported by the significant but low correlations between antibody isotypes, particularly in adults (Chapter 2). We found age-dependent associations between isotypes and strongyle FEC, consistent with IgA and IgG giving the strongest signal of resistance in lambs and adults, respectively. There was also limited evidence for positive associations with body weight, which suggests that immune responses are not strongly driven by condition. Using a much larger, longitudinal dataset we confirmed a previously observed positive association between anti-Tc IgG levels and over-winter survival and showed that this relationship was confined to adult females (Nussey *et al.* 2014; Watson *et al.* 2016). We also detected, for the first time in this system, a positive relationship between anti-Tc IgG levels and annual breeding success in adult males. Generally, we found little evidence of quadratic relationships between anti-Tc antibody levels and fitness, or of strong environment-dependent selection. Our results provide important evidence for age-, sex- and isotype-dependent selection on anti-helminth antibody levels, which may help explain how genetic variation in immune phenotypes is maintained in wild populations.

We found significant negative associations between anti-Tc antibody levels and strongyle FEC, a proxy for parasite burden, which is consistent with levels of these antibodies providing an indication of host resistance function rather than simply reflecting parasite exposure (Hayward *et al.* 2014). However, these associations were age- and isotype-dependent. The significant negative associations observed with all three anti-Tc antibody isotypes and FEC in lambs appeared to be most strongly associated with anti-Tc IgA. Veterinary studies of domestic sheep have demonstrated an important role of the mucosal IgA response in the development of immunity to *T. cirumcincta* in lambs (Stear *et al.* 1995; Strain *et al.* 2002). Rather than actually killing worms, mucosal anti-Tc IgA, which has been shown to be positively correlated with circulating levels of anti-Tc IgA (Henderson & Stear

2006), is involved in reducing female worm growth and fecundity and inhibiting the development of larvae (Stear *et al.* 1995, 2004; Strain *et al.* 2002). Negative associations between FEC and circulating anti-Tc IgA levels have been previously reported in both domestic and Soay sheep lambs (Stear *et al.* 1995, 2004; Coltman *et al.* 2001b; Strain *et al.* 2002). In Soay sheep older than 1 year there was a highly significant negative relationship between anti-Tc IgG and FEC. In adult females, a curvilinear but largely negative association between anti-Tc IgA and FEC was present which was independent of the relationship with anti-Tc IgG. Although studies in domestic ruminants have focussed mainly on young animals (Stear *et al.* 2009), negative associations between anti-Tc IgG levels and FEC or worm burden have been reported for adults (Williams *et al.* 2010; McBean *et al.* 2016) and there is a clear role for IgG in mediating protective immunity against helminths in laboratory mice (Blackwell & Else 2001; McCoy *et al.* 2008). Our results suggest that while anti-Tc IgA levels may represent the best marker of the development of immunity to these parasites during early life, IgG represents the best marker of an adult's long-term ability to cope with persistent exposure and infection with nematode parasites. This highlights the important differences between initial exposure and the development of an immune response to parasitic helminths during early life, and the mature adult immune response which is thought to be more geared towards tolerating infection, wound healing and limiting damage (Allen & Maizels 2011).

There is a strong expectation for immune responses to be tightly linked with condition of the host, with individuals in a better nutritional state more able to invest resources in immunity and therefore to resist infection (van Noordwijk & de Jong 1986; Kraaijeveld & Godfray 1997; Reznick *et al.* 2000). Interestingly, we found evidence for both positive linear and curvilinear relationships between body weight, depending on the age or sex group, and antibody isotype. A number of other studies have shown that body mass and food intake are closely related to immune responses (Siva-Jothy & Thompson 2002; Seppälä & Jokela 2010). However, in a previous study of a smaller sample of adult female Soay sheep, we did not find associations between anti-Tc antibody levels and weight, but did find both positive and negative associations with total and natural antibody measures (Nussey *et al.* 2014). Here we found that although heavier animals tended to have higher anti-Tc IgE levels, adult females with intermediate weights had the highest levels of anti-Tc IgG and IgA. The decline in weight with antibody levels amongst the heaviest individuals may reflect a physiological cost of raised investment in immunity or immunopathology (Råberg, Stjernman &

Hasselquist 2003; Graham *et al.* 2005). Low weight individuals may lack the resources to invest in a strong immune response, meaning that the costs of a raised response are only detectable amongst heavier, good condition animals (Hayward *et al.* 2014).

Selection on anti-Tc antibody levels was highly dependent on isotype, sex and age group: adult females with increased IgG were more likely to survive the next winter whilst adult males were more likely to sire offspring the next spring. This directional selection on anti-Tc IgG was independent of body weight, suggesting it was not mediated by correlated effects on condition. Previous smaller scale studies of this population have found anti-Tc IgG levels to predict over-winter survival, independent of other immune measures and body weight (Nussey *et al.* 2014; Watson *et al.* 2016). This is consistent with studies in other wild systems that have generally found positive associations between immune responses and survival (Saino *et al.* 1997; Christe *et al.* 1998, 2001; Merino *et al.* 2000). There was little evidence for associations between anti-Tc antibody levels and subsequent female fecundity, although adult males with higher anti-Tc IgG were more likely to sire offspring the following year. In Soay sheep, variation in fecundity in adult females is much lower than in males, with 91% of prime-aged ewes (2-6 years old) breeding every year in this dataset, whilst male breeding success is highly variable and skewed towards a handful of individuals (Clutton-Brock & Pemberton 2004). The negative relationship between anti-Tc IgG and FEC in adult sheep implies it captures an important aspect of an individual's ability to resist or tolerate chronic helminth infection. Whatever the underlying causes, the positive relationships between this immune measure and female survival and male breeding success imply it is under largely positive directional selection in this population.

In the preceding chapter, we found differences in the heritabilities of different anti-Tc isotypes (Chapter 2). IgG had the lowest heritability (0.24±0.04), with IgA and IgE having notably higher heritabilities (IgA: 0.58±0.04; IgE: 0.49±0.04) in adults (Figure 2.5/Table 2.1-2.3 from Chapter 2). Classical evolutionary theory predicts that strong directional selection should erode additive genetic variance (Fisher 1930) and that there should be a negative relationship between the strength of directional selection on a trait and its heritability (e.g. Kruuk *et al.* 2000). Our results suggest a pattern across isotypes that fits this prediction, since we found evidence of directional positive selection on anti-Tc IgG but little clear evidence for this in the other two isotypes. In Chapter 2, we also found that anti-Tc IgA levels were influenced by SNPs within a region of chromosome 24 but here found no

association with these SNPs and fitness measures. Although individual SNPs within this QTL explained a substantial amount of the heritability in this immune measure (up to 28%), given the lack of any clear selection on anti-Tc IgA levels in our analyses it is not surprising that none of the SNPs appeared to be under selection.

Unlike a number of experimental studies in wild birds, we found no evidence for a classic trade-off of parasite-specific antibodies and reproductive success (Ilmonen *et al.* 2000; Bonneaud *et al.* 2003; Marzal *et al.* 2007; Gasparini *et al.* 2009). Previous work on the Soay sheep population using an anti-Tc pan-isotype antibody measure, found that adult males with high antibody levels were less likely to sire offspring the following year, and females of high body weight and high antibody levels were also less likely to have a lamb the subsequent year (Hayward *et al.* 2014). In addition, high titres of anti-nuclear antibodies were also associated with reduced breeding success in both adult male and female Soay sheep (Graham *et al.* 2010). This highlights the complex and variable pattern of natural selection that can be observed even on apparently closely functionally related immune measures within the same study population, and reinforces the striking differences observed in this study between anti-helminth antibodies of different isotypes. We measured just one aspect of host immunity, specifically T helper 2-mediated humoral immunity to helminth infection, which may trade-off against other aspects of immunity including T helper 1-mediated responses against protozoan parasites which are known to infect the sheep (Craig *et al.* 2007). In addition, selection does not act on traits in isolation, and will depend on genetic correlations and constraints with other traits, and future work using a multivariate approach will provide us with more information about the evolutionary potential of anti-helminth immune responses (Walsh & Blows 2009).

3.6 Conclusion

We have provided a comprehensive assessment of how circulating antibody levels against a prevalent helminth parasite relate to parasite egg counts, host body weight and host fitness across the lifetimes of thousands of individuals in the wild. Our findings suggest such relationships are strikingly age- and immune measure-dependent. The absence of selection on anti-Tc antibody levels in lambs was surprising, especially given that a previous study found a negative association between strongyle faecal egg counts and annual fitness in lambs

but not in adults (Hayward *et al.* 2011). This pattern may reflect both the greater importance of variation in exposure to helminths for health and fitness in immunologically immature lambs, and a potential switch in the kind of immune response mounted against helminths as the organism matures. Anti-Tc IgG levels were negatively associated with strongyle FEC in adults, showing a switch between protective mechanisms from IgA to IgG between lambs and adults following maturation of the immune system. It has been hypothesised that in early life, when animals are most vulnerable to helminth-associated morbidity, an aggressive immune response to limit worm establishment may develop, while in later life, once immune responses have matured and helminths have established infections, a more tolerant immune phenotype that limits worm fecundity, promotes healing of damaged tissues and prevents immunopathology would be favoured by natural selection (Allen & Maizels 2011; Medzhitov, Schneider & Soares 2012). Progression from resistance to tolerance has been documented in wild voles, where mature males had lower resistance to infection than immature males and parasite burden of mature males was positively associated with body condition (Jackson *et al.* 2014). An age-related switch from resistance to tolerance explain observed shifts in which isotypes best predict FEC and why IgG is under positive selection only in adults, although more detailed immunological study is required to determine if, and how, circulating anti-Tc IgG levels are related to tolerance-polarised immune responses. Numerous studies in the wild, including our own (Chapter 2) have documented low-moderate correlations between immune traits (Matson *et al.* 2006; Nussey *et al.* 2014; Watson *et al.* 2016; Abolins *et al.* 2017). This suggests that studies using a few "catch-all" measures of immunocompetence will not capture the complexity of the immune response and selection acting on it (Demas *et al.* 2011). Our study shows that closely related immune traits may have very different associations with health and fitness measures and suggests that multivariate immune phenotypes must be measured in order to fully understand how genetic variation is maintained in these traits in the wild.

Chapter 4

A pilot study into the causes and consequences of variation in maternally-transferred parasite-specific antibody levels in a wild mammal

4.1 Summary

Maternally-derived antibodies are an important maternal effect that can have a strong influence on offspring growth and fitness. Despite the growing application of quantitative genetics in the wild, a breakdown of maternal antibody transfer into genetic and non-genetic sources of variation has not been carried out. Here, we measured IgA and IgE antibody levels to a common nematode (*Teladorsagia circumcincta*) in wild Soay lambs caught soon after birth as a pilot study into the causes and consequences of variation in maternally-transferred antibody levels for offspring. Where available, we also measured anti-*T. circumcincta* IgA and IgE levels in August when lambs were around 4 months old and producing antibodies endogenously. Neonatal anti-*T. circumcincta* antibody levels were negatively associated with capture age, and positively associated with birth weight. We also found that prime-aged mothers were more likely to transfer higher antibody levels. Quantitative genetic analyses revealed very low additive genetic variance underlying neonatal anti-*T. circumcincta* antibody levels. Maternal and maternal genetic effects explained a considerable proportion of the variance in both traits. In comparison, additive genetic effects explained more of the variance than maternal effects for August antibody levels. Neonatal antibody levels were associated with improved growth and survival in early life. We found that neonatal anti-*T. circumcincta* IgE levels positively predicted both survival to four months old, and weight in August. However, August anti-*T. circumcincta*

IgA levels, but not neonatal antibody levels, were negatively associated with strongyle faecal egg counts, while neither neonatal nor August antibody levels were associated with over-winter survival. Whether neonatal antibody levels are providing direct immunoprotection against parasites, or are just correlated with nutrient quality of the colostrum, is unknown. Although our results are correlational and the mechanisms responsible for associations between anti-*T. circumcincta* antibody levels and growth and survival remain undetermined, this study provides rare evidence quantifying the maternal effects underlying variance in maternal antibody transfer, as well as documenting a fitness benefit of maternally transferred antibodies in the wild.

4.2 Introduction

Offspring phenotype is determined by an individual's genes and the environment they experience, and an important aspect of their environment is their social interactions with conspecifics. A key social interaction is between mothers and offspring, and the direct effect of the mother's phenotype on the phenotype of her offspring, in addition to her genetic contributions, are known as maternal effects. These maternal effects can explain a huge amount of phenotypic variation in offspring and can have important consequences for offspring survival and reproduction (Mousseau & Fox 1998). Genetically-based maternal effects can have important consequences for the evolutionary response to selection, since selection can act on both the mother and offspring. Depending on the correlation with the direct effect of the offspring's genes, maternal genetic effects can either slow down or accelerate the rate of evolution of a character or can lead to evolution in the opposite direction to selection on the offspring trait (Kirkpatrick & Lande 1989; Wolf *et al.* 1998). Maternal effects can also be environmentally determined, with mothers adjusting their phenotype in response to their environment. If these adjustments are adaptive, then non-genetic maternal effects can provide a means for cross-generational phenotypic plasticity, allowing the offspring to be better adapted to the environment (Bernardo 1996; Mousseau & Fox 1998).

Although maternal effects have been documented across a range of taxa, from effects of seed size in plants (Byers, Platenkamp & Shaw 1997), to adult body size in fish (Kruuk *et al.* 2015), maternal effects are widespread and considerably influential in mammals due to the

prolonged association and dependence of offspring on mothers during gestation and lactation (Reinhold 2002). This provides an extended period in which offspring traits could be influenced by maternal condition, as well as other physiological and behavioural mechanisms acting during parental care (Maestripieri & Mateo 2009). One of the best studied parental care behaviours is that of maternal nutritive investment in the young. This nutritional investment can occur both prenatally via the placenta, and postnatally via colostrum and milk, and promotes tissue development and growth of the foetus and neonate and is essential for offspring growth and survival (Langer 2008). In addition, malnutrition in early life has been shown to have long-term effects on offspring physiology into adulthood (De Moura & Passos 2005; McMillen & Robinson 2005).

In addition to transfer of nutrients during lactation, mothers also transfer a number of other immunological components such as lymphocytes, cytokines and immunoglobulins (Butler 1999). Immunoglobulins (or antibodies) are produced by plasma cells in response to infection and are involved in binding specifically to, and neutralising, pathogens or signalling for their uptake and destruction by phagocytes (Murphy 2012). Immunoglobulins are a particularly important component of colostrum and provide protection to offspring against pathogens while their own immune system is developing (Brambell 1970). The diversity and quantity of antibodies transferred to offspring is a function of the mother's exposure to micro- and macroparasites, as well as her immune response to these challenges (Hurley & Theil 2011). This potentially provides a means of transgenerational phenotypic plasticity, in which maternal antibodies prime offspring to the current disease environment (Fox & Mousseau 1998; Agrawal, Laforsch & Tollrian 1999).

Failure of passive transfer of immunoglobulins has been associated with the high neonatal morbidity and mortality rates observed in domestic livestock (Robison *et al.* 1988; Wittum & Perino 1995). In addition, failure of passive transfer has also been associated with reduced growth rates in calves (Robison *et al.* 1988) and birds, where immunoglobulins are deposited in the egg (Buechler *et al.* 2002; Grindstaff 2008). In mice, offspring nursed by immunoglobulin-deficient mothers showed growth retardation and reduced survival (Gustafsson *et al.* 1994). Such benefits to growth may either be due to the reduced costs of mounting an immune response allowing the offspring to concentrate their own resources on growth and development (Grindstaff 2008), or due to the stimulation of cell surface receptors involved in the regulation of neonatal growth by immunoglobulins (Gustafsson *et al.* 1994).

In addition, maternal antibodies have been shown to prevent or delay infection (Dohmae, Koshimizu & Nishimune 1993; Kallio *et al.* 2006). Maternal antibodies provide short-term protection in early life and their persistence in the blood stream varies between species from about 8 weeks in rats (Zhang, Takashima & Hashimoto 1988), to 9 months in human infants (Brambell 1970). Maternal antibodies may continue to impact the immune system of offspring long after the maternal antibodies have been catabolised (Lemke & Lange 1999). For instance, in mice, offspring produce higher antibody titres to antigens to which their mother has been exposed long after the cessation of lactation (Stern 1976; Okamoto *et al.* 1989). However, studies in humans have found that maternal antibodies can have negative effects by suppressing the humoral response to vaccines (Albrecht *et al.* 1977; Björkholm *et al.* 1995; Troisi *et al.* 1997).

Despite the clear benefits of maternal antibody transfer for offspring survival and development, there is huge variation between mothers in levels of antibodies transferred (Zhang *et al.* 1988; Cáceres, Strebel & Sutter 2000; Paoletti *et al.* 2000; Laegreid *et al.* 2002). There is strong evidence that maternal antibody transfer may be genetically determined. Studies of domestic animals have shown high repeatability of IgG levels in colostrum across and within years (Dardillat, Trillat & Larvor 1978; Norman, Hohenboken & Kelley 1981). Genetic markers have also been found for passive transfer of immunity in both mothers and offspring (Laegreid *et al.* 2002; Clawson *et al.* 2004; Rohrer *et al.* 2014). In addition, levels of maternal antibody transferred may be determined by variation in the environment experienced by mothers. For instance, mothers will only be able to pass on antibodies to micro- and macroparasites that they have been exposed to (Lemke & Lange 1999; Gasparini *et al.* 2001). Nutritional status of the mother is also important, since immune function is an inducible defence that is costly to maintain (Klasing 1998). Numerous studies have found that protein restriction reduces both antibody production by the mother (Michalek, Rahman & McGhee 1975) as well as the ability of the neonate to absorb antibodies (Blecha *et al.* 1981). In domestic cows, maternal age or parity has also been positively associated with colostrum IgG levels (Kruse 1970; Muller & Ellinger 1981; Conneely *et al.* 2013).

It is clear from previous studies that mothers vary in the amount of antibodies they transfer to their offspring, due to both genetic and environmental causes, and it is also evident that maternal antibodies are important for offspring growth and survival. The bulk of this

evidence comes from studies focused on humans, rodents, domesticated animals and wild bird systems (Grindstaff *et al.* 2003). Little is known about the role of maternal antibodies in wild mammal populations (Boulinier & Staszewski 2008; Hasselquist, Tobler & Nilsson 2012). There is currently a lack of studies of natural parasite-host systems where detailed knowledge of the dynamics of antibody responses and short and long-term benefits to offspring have been investigated (Hasselquist & Nilsson 2009). Wild populations also offer the opportunity to investigate the relative contributions of genetics and environment in controlling maternal antibody levels, yet no studies exist on the genetics of maternal transfer of antibodies in a wild population. It is highly likely that the levels of maternal antibody are determined by interactions between the maternal genome influencing antibody levels transferred and the offspring genome influencing the amount of antibody absorbed (Grindstaff *et al.* 2003). If maternal antibody transfer is dependent on genetic variation of both mothers and offspring then the underlying genetic correlations would need to be known to accurately predict the evolutionary outcome of selection (Kirkpatrick & Lande 1989; Wolf *et al.* 1998). In addition, maternal antibodies may prime offspring to the current disease environment and therefore may have important implications for disease ecology by altering the proportion of naïve individuals in the population (Grindstaff *et al.* 2003).

In this pilot study we aimed to determine predictors of maternal antibody transfer and the impacts of variation in maternally transferred antibodies for offspring fitness in a wild Soay sheep population (*Ovis aries*). In ruminants, unlike humans and rodents, there is no transfer of immunoglobulins from the mother to the foetus *in utero* due to the complexity of the synepitheliochorial placenta. Since the neonatal ruminant is unable to produce immunoglobulins endogenously until around 4 weeks old, consumption of colostrum has a fundamental role in passive immune transfer (Brambell 1970; Campbell, Siegel & Knowlton 1977). In addition, the non-specific absorption of immunoglobulins from the intestine of neonatal ruminants diminishes rapidly and ceases by 24-36 hours due to gut closure (Brambell 1970; Butler 1999). However, after this time, immunoglobulins continue to be found in milk and may protect the newborn within the lumen of the alimentary tract (Pastoret *et al.* 1998).

The Soay sheep population on St Kilda is an ideal study system for studying causes and consequences of variation in maternal antibody transfer for several reasons. The majority of animals born within the study area are caught and blood sampled within a few days of birth,

and again at four months old and subsequently annually. Individuals are followed throughout their lives and accurate data are collected about the mortality of individuals (Clutton-Brock & Pemberton 2004). In addition, there is strong evidence that early development is important for growth and survival in Soay sheep. For instance, differences in birth weight are positively correlated with weight at four months old and persist until at least 28 months, and heavier born lambs are more likely to survive the neonatal period and their first winter (Clutton-Brock *et al.* 1992). Birth weight is largely influenced by maternal condition, with heavier and prime-aged mothers more likely to have heavier lambs (Clutton-Brock *et al.* 1996; Hayward *et al.* 2013). As such, the condition of mothers appears to have a strong influence on offspring fitness and therefore is strongly linked to how much ewes can invest in their lambs (Clutton-Brock & Pemberton 2004). There are a number of parasitic helminths on the island, including a variety of gastrointestinal strongyle nematodes, largely comprised of the species *Teladorsagia circumcincta*, *Trichostrongylus axei* and *Trichostrongylus vitrinus* (Wilson *et al.* 2004; Craig *et al.* 2006). There is also strong evidence that strongyle parasites are a strong selective agent in this population, influencing winter survival, growth and malnutrition, particularly in lambs (Gulland 1992; Gulland & Fox 1992; Craig *et al.* 2008; Hayward *et al.* 2011). Exposure of lambs to strongyle parasites is likely to begin early in life, since Soay lambs will nibble grass by five days old and are typically weaned by 3 months (Clutton-Brock & Pemberton 2004). A number of studies have shown associations of protection against *T. circumcincta* with antibody isotypes IgA and IgE in domestic sheep (Stear *et al.* 1995; Stear, Park & Bishop 1996; Murphy *et al.* 2010). In the Soay sheep, strongyle FEC is negatively associated with both IgA antibodies against crude L3 *T. circumcincta* antigens and a cross-isotype assay of antibodies against crude adult *T. circumcincta* antigens (Coltman *et al.* 2001b; Hayward *et al.* 2014). In Chapter 3, we also found that high strongyle faecal egg counts were associated with lower anti-*T. circumcincta* IgA in lambs and lower anti-*T. circumcincta* IgG in adults. Therefore, it is likely that helminth-specific maternally transferred antibodies could be particularly important in early life for Soay lambs.

In this chapter, we aim to investigate the factors associated with neonatal levels of anti-*T. circumcincta* IgA and IgE, such as capture age of the neonate, birth weight, sex and twin status as well as the mother's age. As neonatal lambs are caught close to birth, all antibodies in plasma samples taken at this time would be maternally-derived rather than endogenously produced. We also looked at predictors of anti-*T. circumcincta* IgA and IgE in August when

lambs are around 4 months old. At this time point, lambs are able to produce their own antibodies and sucking bouts are rare (Clutton-Brock & Pemberton 2004; McRae *et al.* 2015). We next performed quantitative genetic analyses to partition the phenotypic variance in neonatal antibodies levels and August antibody levels into additive genetic, maternal genetic and environmental sources of variation. In Chapter 2, we did not include neonatal samples and maternal effects underlying lamb August antibody levels were not split into their underlying genetic and environmental components. Finally, we investigated whether variation in neonatal or August antibody levels were associated with lamb weight, strongyle FEC levels and over-winter survival. Investigating antibody levels at these two ages presents an additional opportunity to compare and contrast the causes and consequences of variation in immunity at two time points during early life. This chapter forms a pilot study into the causes and consequences of variation in maternal antibody levels in neonatal Soay sheep, and forms the basis for the collection of a much larger dataset that is analysed in Chapter 5.

4.3 Methods

4.3.1 Study population

The Soay sheep is a primitive breed of domestic sheep that was isolated on the island of Soay in the remote St Kilda archipelago several millennia ago, and has been living under unmanaged conditions and evolving under natural selection over that period (Clutton-Brock & Pemberton 2004). In 1932, just over 100 Soay sheep were moved to the larger island of Hirta after the evacuation of all human residents. Approximately a third of the current population of Hirta Soay sheep live in the Village Bay area of Hirta, and these individuals have been the subject of a long-term study since 1985 (Clutton-Brock & Pemberton 2004). In April each year around 95% of all lambs born in this area are caught and individually tagged, weighed and bled. Each August as many sheep as possible from the study population are re-captured using temporary traps (Clutton-Brock & Pemberton 2004). At capture in August, animals are weighed and blood and faecal samples are collected. Whole blood samples are collected into heparin tubes, centrifuged at 3000 r.p.m. for 10 minutes, and plasma removed and stored at -20°C. Strongyle faecal egg count (FEC) is estimated from faecal samples as the number of eggs per gram using a modified McMaster technique (Gulland & Fox 1992). Three species: *T. circumcincta*, *Trichostrongylus axei* and

Trichostrongylus vitrinus contribute the majority of the strongyle eggs counted (Craig *et al.* 2006).

In this analysis we included lambs which were caught between 1997 and 2007 and had plasma samples taken in April just after birth. When available, we also analysed plasma samples taken in August from these same lambs at around 4 months old. No April samples or data were collected in 2001 due to Foot and Mouth disease precautions. Mortality was determined by censuses of the population and by mortality searches. In this study we included two survival periods, neonatal survival and first winter survival. Neonatal mortality was defined as lambs dying before the 1st August of their year of birth, while first winter mortality was defined as individuals dying between 1st August and 30th April the following year.

4.3.2 Laboratory methods

IgA and IgE activity against antigens of the third larval stage of *T. circumcincta* was measured using optimised direct and indirect ELISAs, respectively. We used *T. circumcincta* L3 somatic antigen, provided by the Moredun Research Institute, as the capture antigen for both assays diluted to 2µg/ml in 0.06M carbonate buffer at pH 9.6. 50µl of the diluted capture antigen was added to each well of a Nunc-immuno 96-microwell plate, which was covered and incubated at 4°C overnight. After washing the wells three times in Tris-buffered saline-Tween (TBST) using a plate washer, 50µl of the Soay sheep plasma sample diluted to 1:100 was added to each well. The plates were then covered and incubated at 37°C for 1 hour. They were then washed five times with TBST, and 50µl per well of rabbit anti-sheep detection antibody conjugated to horseradish peroxidase (HRP) (AbD Serotec AHP949P) was added to the anti-*T. circumcincta* IgA assay. For the anti-*T. circumcincta* IgE assay, 50µl per well of anti-sheep IgE (mouse monoclonal IgG1, clone 2F1, provided by the Moredun Research Institute) diluted 1:100 was added, followed by 1 hour incubation at 37°C, five washes with TBST, and then 50µl per well of goat anti-mouse IgG1-HRP detection antibody (AbD Serotec STAR132P) was added diluted to 1:8000 in TBST. All plates were then incubated at 37°C for 1 hour. Plates were then washed five times with TBST, and 100µl of SureBlue TMB 1-Component microwell peroxidase substrate (KPL) was added per well and left to incubate for 5 minutes in the dark at 37°C. Reactions were

stopped by adding 100µl per well of 1M hydrochloric acid, and optical densities (OD) were read immediately at 450nm using a Thermo Scientific GO Spectrophotometer.

All results were recorded as OD values. All assays were duplicated with randomisation of the location of each sample between plates. Duplicate sample ODs were compared and removed if the coefficient of variation was > 0.2 and the difference between ODs was greater than 0.2. We also checked the correlation of ODs across duplicate plates and re-ran both plates if r < 0.8.To reduce error due to within-plate variation, we included three sample-free wells (50µl TBST) as negative controls, and three wells of positive controls of 50µl of purified lymph diluted 1:100 from domestic sheep challenged twice with *T. circumcincta* (provided by the Moredun Research Institute). For subsequent analyses, the means of sample duplicates minus the average of the six negative control ODs were used. Of a total of 717 lambs, the number of samples that failed quality control per assay was 23 for neonatal anti-*T. circumcincta* IgA, 27 for neonatal anti-*T. circumcincta* IgE, 46 for August anti-*T. circumcincta* IgA, and 46 for August anti-*T. circumcincta* IgE. Distributions of antibody levels are shown in Figure 4.1.

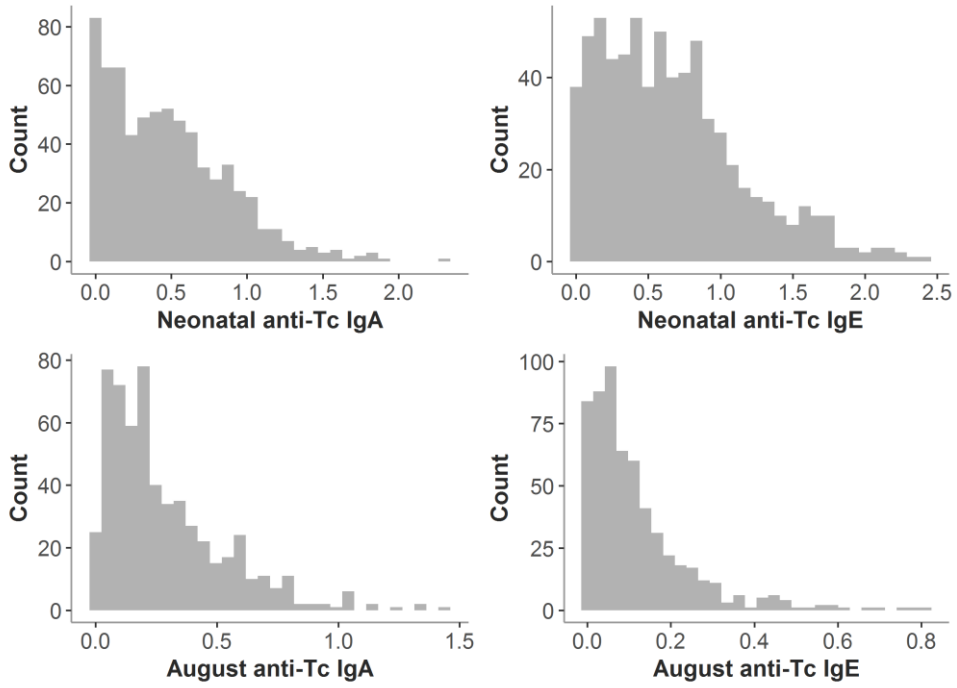

Figure 4.1. Histograms of neonatal and August anti-*Teladorsagia circumcincta* IgA and IgE levels in Soay lambs.

4.3.3 Statistical analyses

For the analysis we restricted the dataset to all animals caught within five days of birth for three reasons. Firstly, to accurately estimate birth weight from capture weight in the period where growth rate is linear (Robertson *et al.* 1992), secondly due to a sharp decline of antibodies in older lambs (see Results), and finally to account for low sample sizes in older capture age groups (n = 63). In total we had a sample size of 628 lambs from 304 mothers who had anti-*T. circumcincta* ("anti-Tc") IgA and IgE levels in April, and 531 lambs from 271 mothers who had anti-*T. circumcincta* IgA and IgE levels in August. Of the lambs in the dataset 47% were females and 23% were twins. 8% of lambs did not survive to August and 46% did not survive their first winter.

Models of antibody levels

We examined potential causes of variation in neonatal and August anti-Tc IgA and IgE levels using linear mixed effects models (LMM) with antibody levels as a response variable in the "lme4" package in R 3.3.0 (Bates *et al.* 2015; R Core Team 2016). These models included maternal identity and year as random effects to account for repeated measures and variation between maternal siblings and years. We first fitted a model containing fixed effects comprising offspring and maternal traits that had previously been identified as important predictors of lamb condition and fitness (Clutton-Brock *et al.* 1992, 1996; Hayward *et al.* 2013). We then simplified the model by step-wise deletion, sequentially removed the fixed effects with the lowest (non-significant) t values and determined statistical significance using likelihood ratio tests until a minimal model containing only significant fixed effects was left. All deleted non-significant terms were then re-tested against this base model using the same criteria.

For the neonatal antibody models we included lamb sex, twin status, birth weight, capture age of the lamb, and maternal age (as a quadratic function) as fixed effects. A variety of functions of capture age were fitted to the models to determine which best explained variation. Linear and quadratic functions of capture age were compared with threshold models with a single threshold at day 1 to 4 (following Berman, Gaillard & Weimerskirch 2009). The best model of capture age was chosen based on the lowest AIC value, unless the difference in AIC value between the two best fitting models was < 2, in which case the most parsimonious model was chosen. Since Soay lambs were not caught at birth, birth weight

was estimated by taking the residuals from a linear model with a quadratic function of capture age on capture weight.

Potential causes of variation in August anti-Tc IgA and IgE levels were investigated using similar models with August antibody levels as response variables. Although the random effects were the same, the fixed effects differed and included lamb sex, twin status, August weight, age in days and maternal age (as a quadratic function).

Animal model

We next fitted quantitative genetic "animal models" in order to determine the maternal genetic and additive genetic basis of neonatal and August antibody levels in ASReml-R 3.0 (Butler *et al.* 2009). The pedigree used was constructed using maternities and paternities assigned with 315 unlinked single nucleotide polymorphisms (linkage disequilibrium $r^2 < 0.05$) with a minor allele frequency > 0.4 using the R library *sequoia* (Huisman 2017). This pedigree included all cohorts from 1985-2015 and includes 8221 individuals with 7142 maternities and 5456 paternities.

Univariate animal models were fitted for each of the four neonatal and August anti-Tc antibody measures. Fixed effects were included as determined from the LMM analyses for the neonatal antibody levels but excluding birth weight due to potential genetic correlations between these traits (Wilson 2008). For the August antibody levels the fixed effects included were sex, twin status and age in days. The random effects included the additive genetic component, maternal genetic and environment components, and birth year. Significance of random effects was determined by dropping each random effect from a model containing all random effects and performing a likelihood ratio test on the two log likelihoods.

The proportion of the phenotypic variance explained by each random effect was estimated as the ratio of the relevant variance component to total phenotypic variance, defined as the sum of all variance components. The direct heritability of each measure was determined as the ratio of the additive genetic variance to the total phenotypic variance. Where the maternal genetic and additive genetic components were significant, we additionally estimated the direct-maternal genetic covariance. The direct-maternal genetic correlation was determined

by dividing the direct-maternal genetic covariance by the product of the square roots of the maternal genetic variance and the additive genetic variation. Standard errors of ratio components were calculated using the pin function from the R package "nadiv" (Wolak 2012).

Models of offspring growth, parasite burden and survival

We next looked at associations between neonatal and August anti-Tc IgA and IgE levels and four health and fitness related traits for the lamb: neonatal survival (survival to 4 months), August weight, August strongyle FEC and first winter survival. All models were run using the "lme4" package in R 3.3.0 (Bates *et al.* 2015; R Core Team 2016). Analyses of survival were performed using generalised linear mixed models (GLMMs) with a binomial error structure, while analyses of August weight and FEC used LMMs. August FEC measures were log(x+100) transformed prior to analysis to normalise their distribution. All models included year and maternal identity as random effects for the same reasons as stated above, but the fixed effects structure differed. We included fixed effect terms that are well-established predictors of FEC, weight and survival from previous studies. In the August weight and FEC models, twin status, sex, age in days and maternal age (linear and quadratic) were common to both models (Clutton-Brock & Pemberton 2004). In addition, birth weight and August FEC were added as fixed effects in the August weight model, while August weight was included in the August FEC model (Clutton-Brock et al. 1992; Hayward et al. 2014). In the neonatal and first winter survival models the fixed effect terms of twin status, sex and maternal age (linear and quadratic) were common to both models (Clutton-Brock *et al.* 1992; Hayward *et al.* 2013). In addition, birth weight was included in the neonatal survival model, while August FEC and August weight were included in the first winter survival model (Clutton-Brock *et al.* 1992; Gulland 1992; Hayward *et al.* 2011). First we fitted a model containing all fixed effects for each of the response variables which were kept in the models as previously established predictors of FEC, weight and survival measures in the sheep. To each model, the four antibody isotype measures - neonatal anti-Tc IgA, neonatal anti-Tc IgE, August anti-Tc IgA and August anti-Tc IgE levels - were added. Due to the strong association between neonatal anti-Tc antibodies and capture age, neonatal anti-IgA and IgE levels were corrected for capture age by taking residuals from a linear model for IgE and from a threshold model at day 1 for IgA (which were the best fitting functions of capture age, see Results). We then tested whether, and which, antibody measures predicted each response variable, independent of the other explanatory variables, by sequentially

deleting antibody terms from the model as described above until only those that were significant remained. All deleted non-significant terms were then re-tested against the minimal model using the same criteria.

4.4 Results

4.4.1 Predictors of neonatal antibody levels

Neonatal anti-Tc IgA and IgE levels showed considerable variation and there was a weak positive correlation between the measures ($r = 0.33$, $t_{626} = 8.64$, $p < 0.001$, Figure 4.2). Capture age and birth weight of the lamb, as well as maternal age, were associated with neonatal antibody levels. IgE levels in neonates declined linearly with age at capture (ΔAIC = +1.796 to next best model), but the pattern for IgA was best described by a threshold model with a peak at day 1 before declining (ΔAIC = -29.009 to next best model; Table 4.1, Figure 4.3). Birth weight was also a predictor of both antibody levels, with heavier lambs having higher antibody levels of both isotypes (Table 4.1, Figure 4.3). In the neonatal anti-Tc IgE model there was a curvilinear association of mother's age with this isotype, in which offspring of prime-aged mothers had higher antibody levels (Table 4.1, Figure 4.3). In contrast, after inclusion of birth weight as a fixed effect in the neonatal anti-Tc IgA model, twins were associated with higher antibody levels and a negative linear association was seen with mother's age (Table 4.1, Figure 4.3). There was no effect of lamb sex on neonatal antibody levels of either isotype. Maternal identity explained 40% and 43% of the variance in both neonatal anti-Tc IgA and IgE levels respectively, suggesting that mothers are consistent in the levels of antibodies they provide.

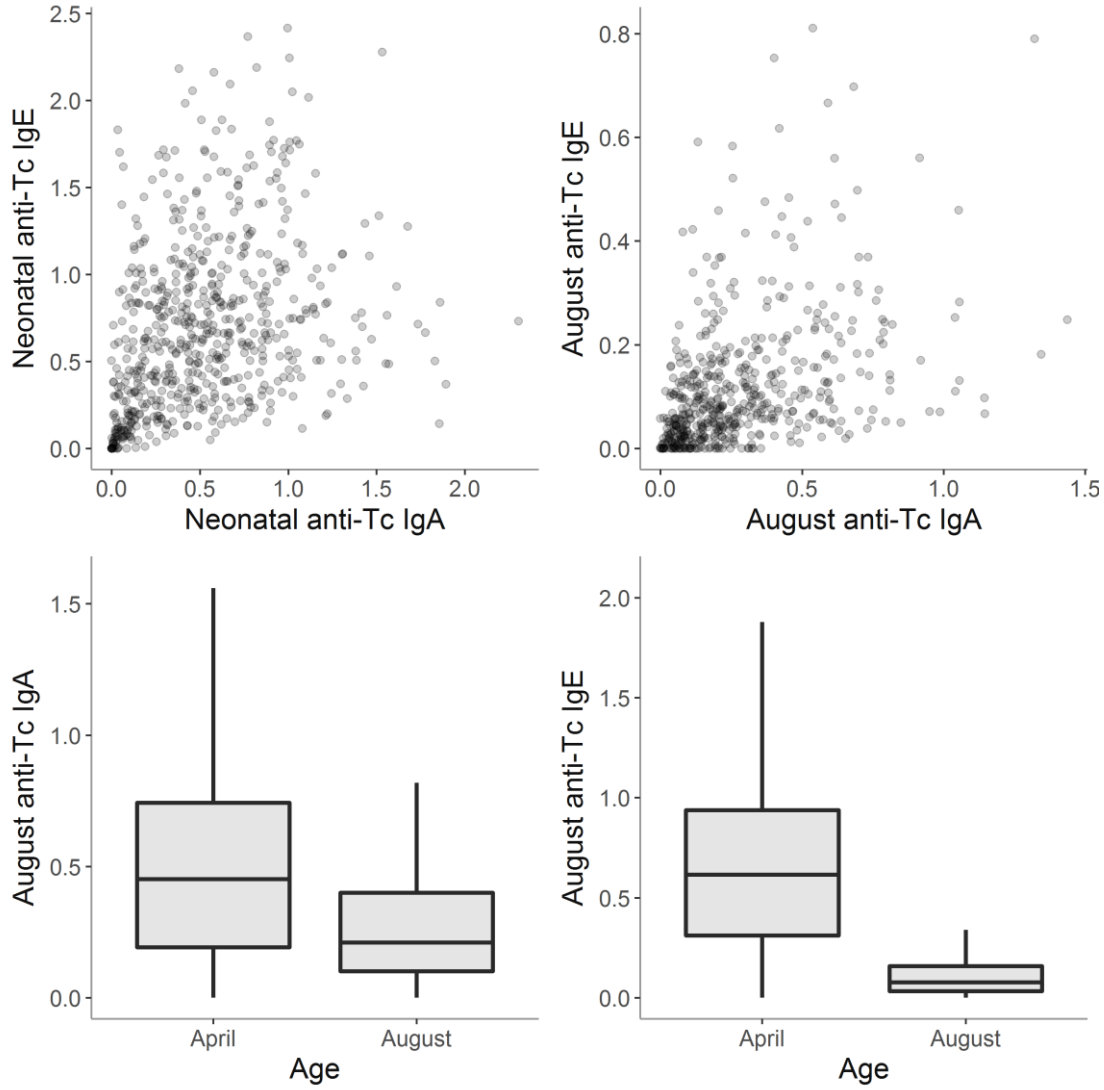

Figure 4.2. Scatterplots of raw data showing correlations between anti-*Teladorsagia circumcincta* IgA and IgE levels in neonates in April and in 4 month old lambs in August. Boxplots show comparisons between the levels of anti-*T. circumcincta* IgA and IgE levels between April (neonatal) and August. Boxes show the median and the interquartile range (IQR) with whiskers extending from the hinges to values no further than 1.5*IQR from the hinge (outliers not shown).

Table 4.1. LMM results of the final minimal model showing the effects of lamb and maternal characteristics on neonatal anti-*T. circumcincta* IgA and IgE levels. Likelihood ratio test (LRT) results quoted are for the relevant terms when added back to the final model. Estimates quoted for random effects are variances. Significant results are shown in bold.

	April IgA model					**April IgE model**				
variables	estimate	SE	LRT	d.f.	p-value	estimate	SE	LRT	d.f.	p-value
fixed effects										
twin	0.105	0.040	7.111	1	**0.008**					
sex (male)										
capture age						-0.100	0.014	47.903	1	**<0.001**
≤1 day	0.223	0.065	11.619	1	**<0.001**					
>1 day	-0.171	0.011	209.670	1	**<0.001**					
birth weight	0.144	0.030	22.033	1	**<0.001**	0.211	0.035	35.538	1	**<0.001**
maternal age	-0.030	0.006	25.452	1	**<0.001**	0.082	0.029			
maternal age^2						-0.006	0.002	6.203	1	**0.013**
random effects										
maternal ID	0.045					0.094				
year	0.010					0.020				
residual	0.058					0.103				
dropped fixed effects										
sex (male)			2.611	1	0.106			0.025	1	0.875
twin								1.828	1	0.176
maternal age^2			0.007	1	0.935					

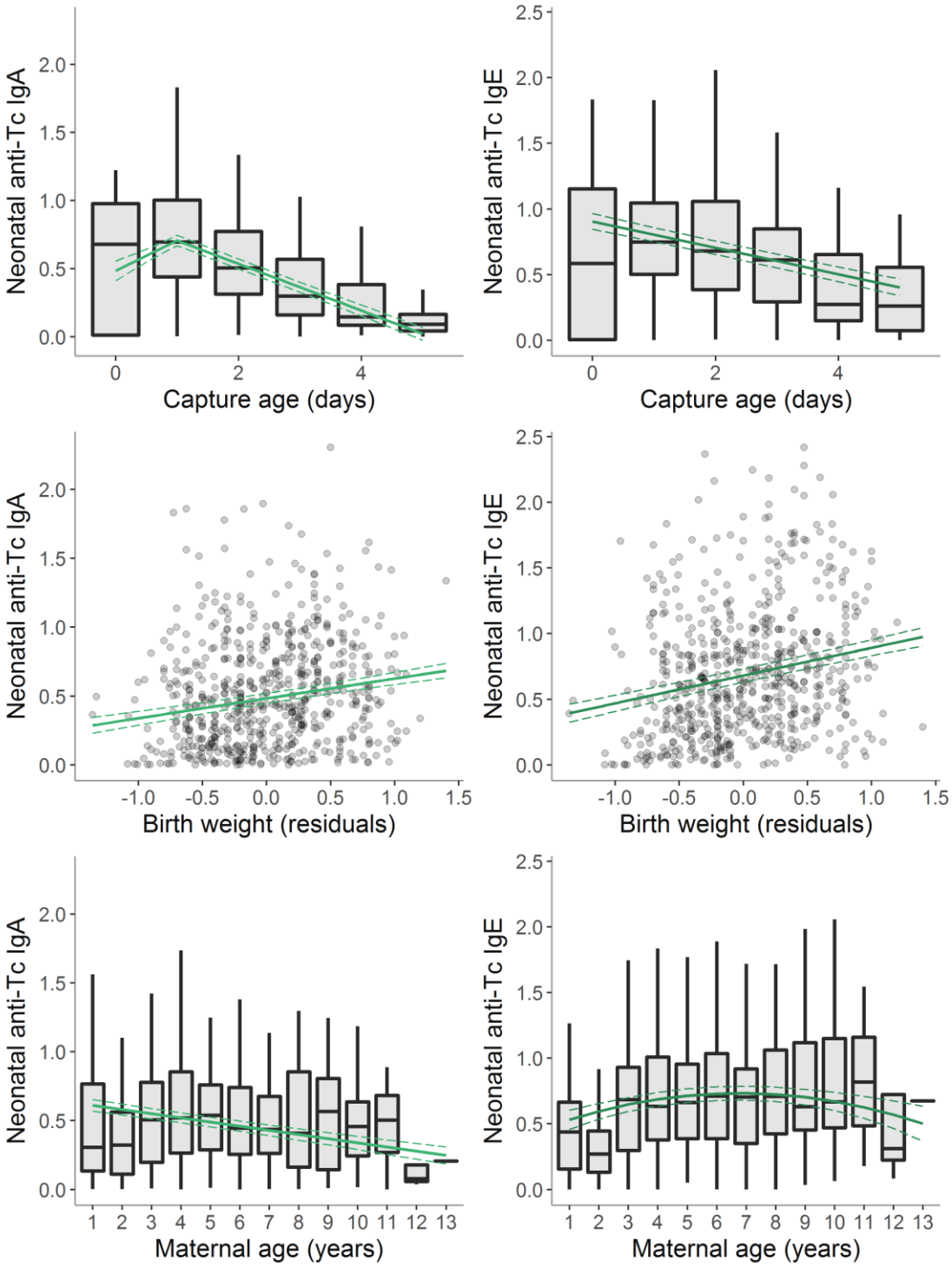

Figure 4.3. The associations between capture age, birth weight and maternal age on neonatal anti-*Teladorsagia circumcincta* IgA and IgE levels. Plots show raw data with LMM predictions based on the final minimal model. LMM predictions are estimated at average values for all continuous fixed effects in the minimal model, and singletons for the IgA model. Boxes show the median and the interquartile range (IQR) with whiskers extending from the hinges to values no further than 1.5*IQR from the hinge (outliers not shown).

4.4.2 Predictors of August antibody levels

Antibody levels declined on average from April to August (IgA: $t_{528} = 12.82$, $p < 0.001$; IgE: $t_{525} = 27.18$, $p < 0.001$, Figure 4.2). August IgA and IgE were correlated with each other ($r = 0.41$, $t_{529} = 10.38$, $p < 0.001$, Figure 4.2). However, neonatal antibody levels were not significantly correlated with August antibody levels of the same isotype (IgA: $r = 0.02$, $t_{527} = 0.55$, $p = 0.58$; IgE: $r = -0.06$, $t_{524} = -1.32$, $p = 0.19$). Age in days was positively associated with both August antibody isotypes, while males had lower August antibody levels than females (Table 4.2, Figure 4.4). Twins had higher August IgA levels than singletons, while there was a positive linear association with weight for August IgE (Table 4.2). Compared to the neonatal models, the proportion of variance explained by maternal identity dropped to 23% and 17% for IgA and IgE respectively, and maternal age was not a significant predictor of either isotype levels (Table 4.2).

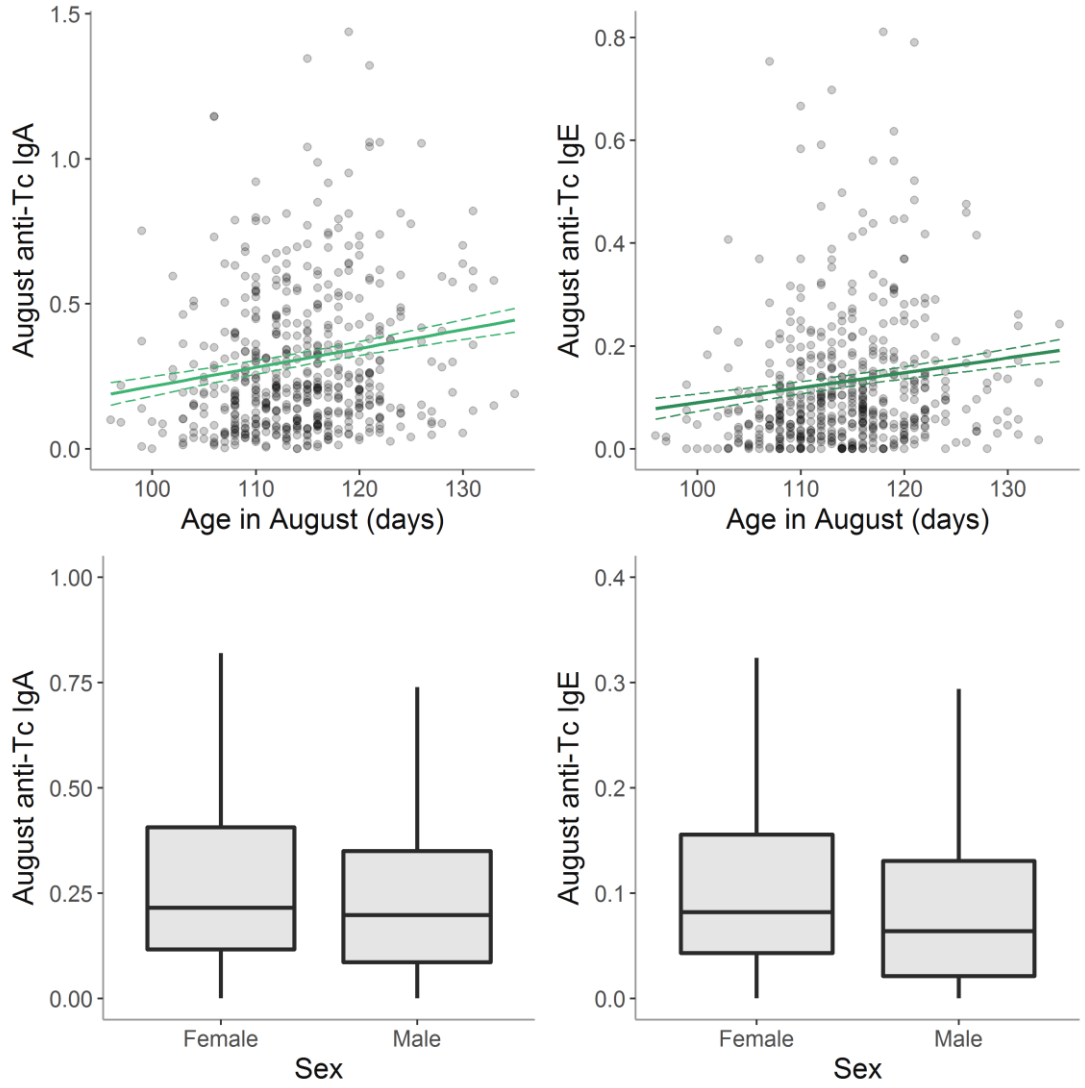

Figure 4.4. Associations between age and sex and August anti-*Teladorsagia circumcincta* IgA and IgE levels. The scatterplots show raw data and LMM predictions for the association between age in days and August anti-*T. circumcincta* IgA and IgE levels. LMM predictions are estimated for female singletons with average values for all continuous fixed effects in the minimal model. Boxplots show comparisons between the raw levels of August anti-*T. circumcincta* IgA and IgE between female and male lambs. Boxes show the median and the interquartile range (IQR) with whiskers extending from the hinges to values no further than 1.5*IQR from the hinge (outliers not shown).

Table 4.2. LMM results of the final minimal model showing the effects of lamb and maternal characteristics on anti-*T. circumcincta* IgA and IgE levels at four months old. Likelihood ratio test (LRT) results quoted for dropped fixed effects are for these terms when singly added back to the final model. Estimates quoted for random effects are variances. Significant results are shown in bold.

	August IgA model					**August IgE model**				
variables	estimate	SE	LRT	d.f.	p-value	estimate	SE	LRT	d.f.	p-value
fixed effects										
twin	0.056	0.026	4.563	1	**0.033**					
sex (male)	-0.066	0.020	11.075	1	**<0.001**	-0.023	0.011	4.277	1	**0.039**
age (days)	0.007	0.002	14.752	1	**<0.001**	0.003	0.001	10.543	1	**0.001**
August weight						0.005	0.002	3.957	1	**0.047**
random effects										
maternal ID	0.013					0.003				
year	0.002					<0.001				
residual	0.042					0.013				
dropped fixed effects										
maternal age			0.011	1	0.918			0.132	1	0.716
maternal age^2			0.046	1	0.977			0.264	2	0.877
August weight			0.042	1	0.839					
twin								0.442	1	0.506

4.4.3 Animal model

There was evidence for a significant but low direct additive genetic effect on neonatal anti-*T. circumcincta* IgE but not IgA antibody levels (IgA $h^2 = 0.094 \pm 0.078$ SE; IgE $h^2 = 0.140 \pm 0.073$ SE). There were considerable maternal effects explaining neonatal anti-*T. circumcincta* antibody levels, with maternal identity explaining 36% and 37% of the phenotypic variance in anti- *T. circumcincta* IgA and IgE levels respectively (Table 4.3, Figure 4.5). Maternal genetic effects explained a significant proportion of the variance in both neonatal antibody levels, and the proportion of phenotypic variance explained by maternal genetic variance was 0.18±0.10 SE in IgA and 0.34±0.10 SE in IgE. This maternal genetic component explained 51% and 93% of the maternal effect for IgA and IgE respectively (Table 4.3, Figure 4.5). Estimates of the direct-maternal genetic correlation were low and negative for IgE ($r_{am} = -0.159 \pm 0.330$ SE), but this correlation was not significantly different from 0 based on these standard errors and did not significantly improve model fit ($\chi^2_{(1)} = 0.205$, $p = 0.650$).

There was evidence for a significant direct additive genetic and maternal genetic effects on August anti-*T. circumcincta* IgA levels but neither were significant for IgE levels (Table 4.4, Figure 4.5). Maternal effects explained less of the variance in August antibody levels compared to neonatal antibody levels (at 16% and 12% of the phenotypic variance in anti-*T. circumcincta* IgA and IgE levels respectively). Combined maternal effects in August explained less than the direct additive genetic effects (IgA $h^2 = 0.279 \pm 0.117$ SE; IgE $h^2 = 0.147 \pm 0.110$ SE). Estimates of the direct-maternal genetic correlation were low and negative for IgA ($r_{am} = -0.089 \pm 0.481$ SE), but this correlation was not significantly different from 0 based on these standard errors and did not significantly improve model fit ($\chi^2_{(1)} = 0.027$, $p = 0.869$).

Table 4.3. Variance component estimates and their associated ratios for models of neonatal anti-*Teladorsagia circumcincta* IgA and IgE levels measured in St. Kilda Soay sheep. Variances reported are the additive genetic variance (V_A), maternal genetic variance (V_{MG}), maternal environment variance (V_{ME}), year variance (V_{YEAR}), and residual variance (V_R). Included are the raw variance component estimates ('Est') and the proportion of the total phenotypic variance explained by the term ('Prop') and their associated standard errors in brackets. The significance of random effects was determined by dropping each random effect from a model containing all random effects and performing a likelihood ratio test ('LRT' and 'p-value') on the two log likelihoods. [B] indicates where variance components went to boundary in the model. Significant results are shown in bold.

	Neonatal anti-Tc IgA n = 632					**Neonatal anti-Tc IgE** n = 628				
	Est	**Prop**	**LRT**	**df**	**p-value**	**Est**	**Prop**	**LRT**	**df**	**p-value**
V_A	0.011 (0.009)	0.094 (0.078)	1.868	1	0.172	0.033 (0.017)	0.140 (0.073)	**7.716**	**1**	**0.005**
V_{MG}	0.022 (0.012)	0.181 (0.095)	**4.108**	1	**0.043**	0.080 (0.025)	0.344 (0.099)	**12.907**	**1**	**0.000**
V_{ME}	0.021 (0.010)	0.177 (0.087)	**4.171**	1	**0.041**	0.006 (0.018)	0.024 (0.079)	0.080	1	0.778
V_{YEAR}	0.013 (0.007)	0.107 (0.051)	**56.218**	1	**<0.001**	0.027 (0.014)	0.116 (0.054)	**61.036**	**1**	**<0.001**
V_R	0.053 (0.008)	0.441 (0.072)	-	-	-	0.088 (0.014)	0.377 (0.066)	-	-	-

Table 4.4. Variance component estimates and their associated ratios for models of August anti-*Teladorsagia circumcincta* IgA and IgE levels measured in St. Kilda Soay sheep. Variances reported are the additive genetic variance (V_A), maternal genetic variance (V_{MG}), maternal environment variance (V_{ME}), year variance (V_{YEAR}), and residual variance (V_R). Included are the raw variance component estimates ('Est') and the proportion of the total phenotypic variance explained by the term ('Prop') and their associated standard errors in brackets. The significance of random effects was determined by dropping each random effect from a model containing all random effects and performing a likelihood ratio test ('LRT' and 'p-value') on the two log likelihoods. [B] indicates where variance components went to boundary in the model. Significant results are shown in bold.

	August anti-Tc IgA n = 531					**August anti-Tc IgE** n = 531				
	Est	**Prop**	**LRT**	**df**	**p-value**	**Est**	**Prop**	**LRT**	**Df**	**p-value**
V_A	0.016 (0.007)	0.279 (0.117)	**10.001**	**1**	**0.002**	0.002 (0.002)	0.147 (0.110)	2.442	1	0.1181
V_{MG}	0.010 (0.004)	0.164 (0.065)	**4.315**	**1**	**0.038**	<0.001 (0.001)	0.025 (0.072)	0.139	1	0.7094
V_{ME}	<0.001 (<0.001)[B]	<0.001 (<0.001)[B]	NA	NA	NA	0.002 (0.001)	0.095 (0.076)	2.034	1	0.1538
V_{YEAR}	0.003 (0.002)	0.045 (0.029)	**11.143**	**1**	**0.001**	<0.001 (<0.001)	0.022 (0.019)	3.759	1	0.0525
V_R	0.030 (0.005)	0.511 (0.099)	-	-	-	0.011 (0.002)	0.711 (0.094)	-	-	-

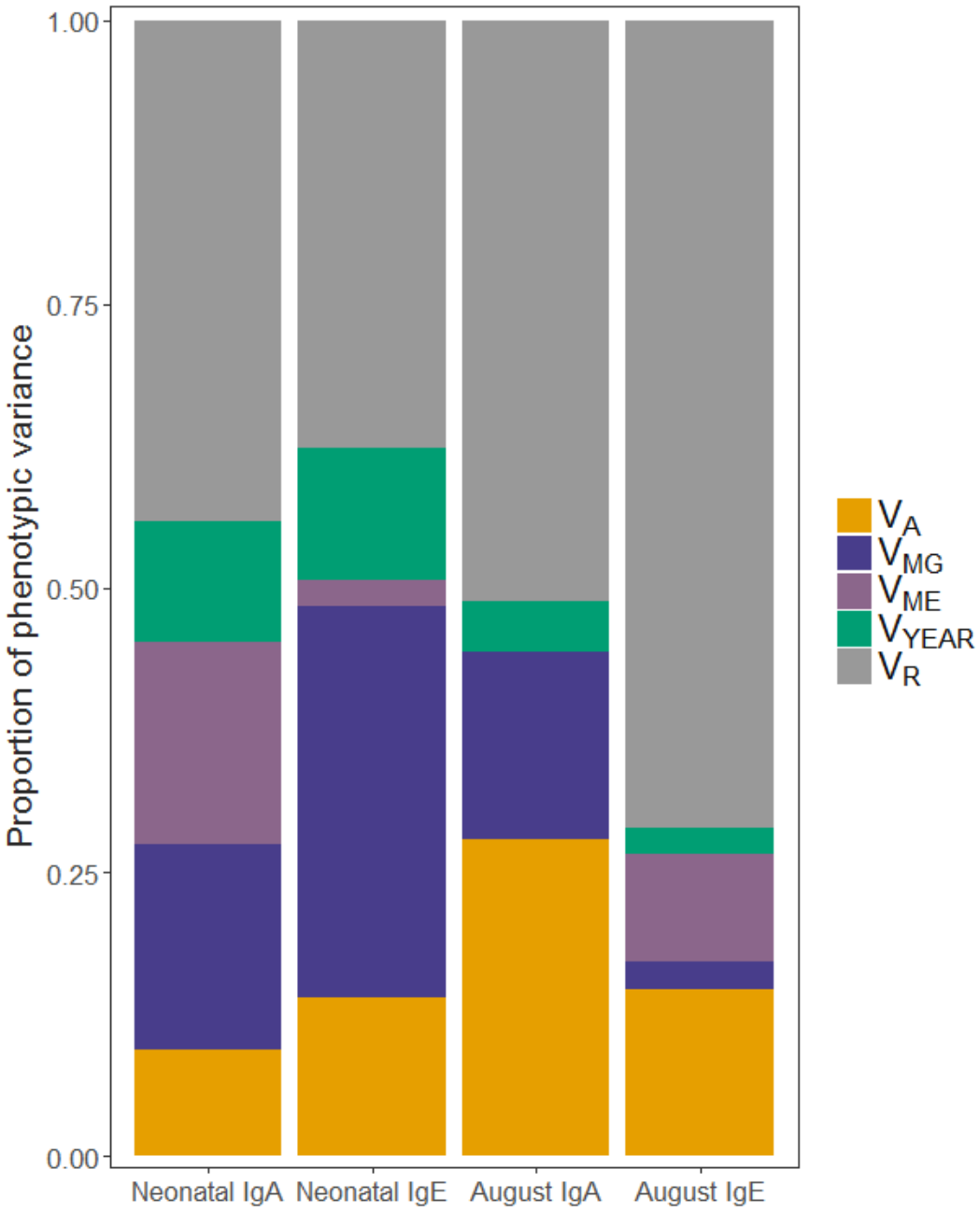

Figure 4.5. The proportion of phenotypic variance explained by different random effects in univariate animal models for neonatal and August anti-*Teladorsagia circumcincta* IgA and IgE levels in wild Soay sheep. Random effects include the following variance components: additive genetic (V_A), maternal genetic (V_{MG}), maternal environment (V_{ME}), birth year (V_{YEAR}) and the residual error (V_R).

4.4.4 Consequences of variation in neonatal antibody levels

There was a positive linear effect of neonatal IgE levels on lamb August weight, independent of birth weight, sex, twin status and age in days (Table 4.5, Figure 4.6). When IgE was not in the model, neonatal IgA levels significantly positively predicted lamb August weight ($\chi^2_{(1)}$ = 5.598, p = 0.018; Figure 4.6), suggesting that both isotypes are explaining shared variance. However, there was no effect of neonatal IgA or IgE levels on August FEC (Table 4.5). August antibody levels had no effect on lamb August weight after accounting for neonatal antibody levels, but there was a marginal negative effect of August IgA on August FEC (Table 4.5). When August IgA was not in the model, August IgE levels were also negatively associated with August FEC ($\chi^2_{(1)}$ = 4.530, p = 0.033).

There was also a positive effect of neonatal IgE levels on neonatal survival, with lambs with higher neonatal IgE levels more likely to survive to August independent of birth weight, sex and twin status (Table 4.6, Figure 4.6). When IgE was not in the model, neonatal IgA levels had a marginally non-significant effect on neonatal survival ($\chi^2_{(1)}$ = 3.673 p = 0.055). However, there was no significant effect of variation in either neonatal or August antibody levels on first winter survival (Table 4.6).

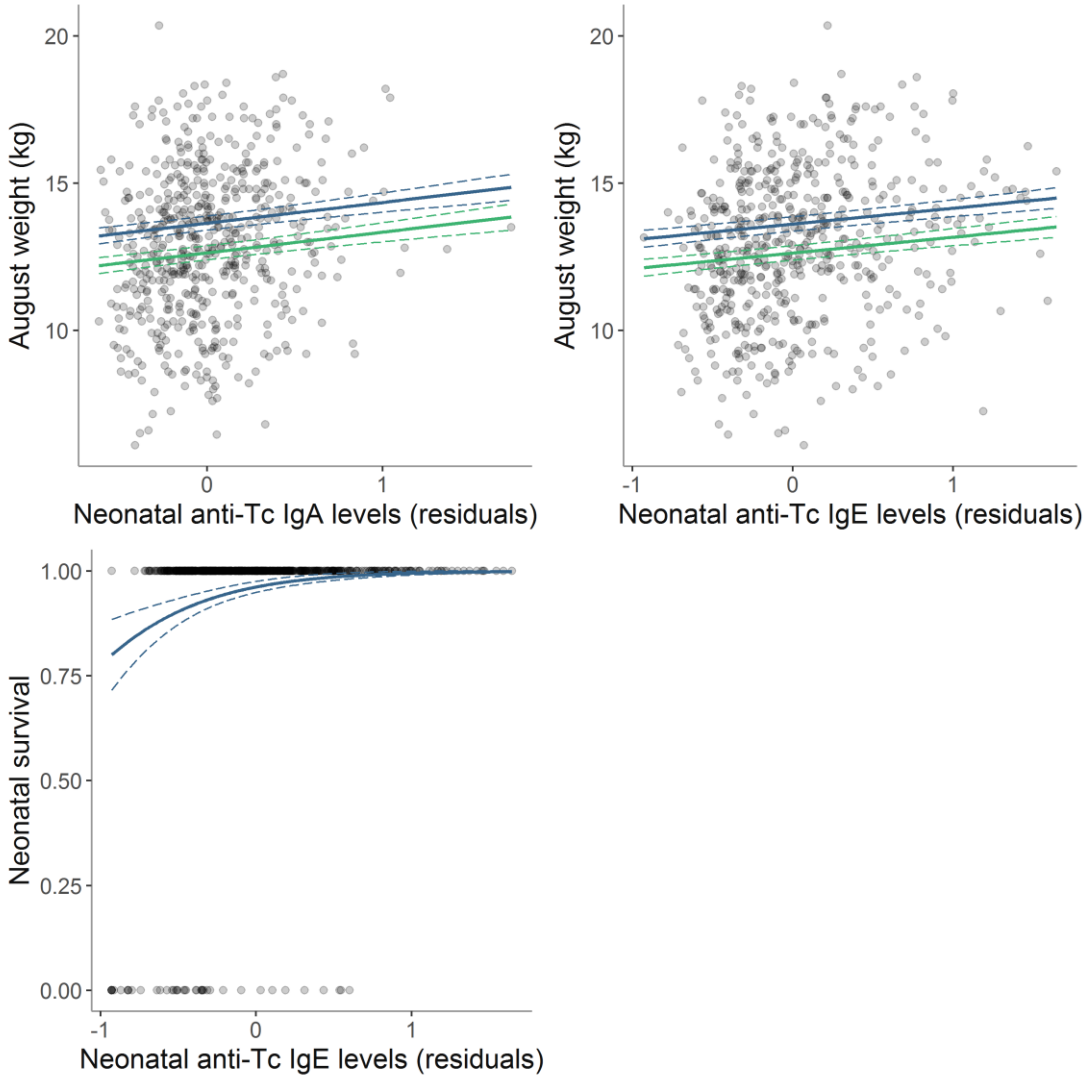

Figure 4.6. Associations between neonatal anti-*T. circumcincta* IgA and IgE levels on neonatal survival to four months old, and on weight in August. August weight plots show LMM prediction lines for female (green) and male (blue) singletons and the neonatal survival plot shows GLMM prediction for male singletons. Neonatal anti-*T. circumcincta* antibody levels are corrected for capture age by taking residuals from a linear model for IgE and from a threshold model at day 1 for IgA.

Table 4.5. LMM and GLMM results of the final minimal model showing the effects of neonatal and August anti-*T. circumcincta* IgA and IgE levels on weight and strongyle FEC respectively of lambs at four months old. Likelihood ratio test (LRT) results quoted for dropped fixed effects indicate these terms when singly added back to the final model. Estimates quoted for random effects are variances. Significant results are shown in bold.

	August weight model					**August FEC model**				
variables	estimate	SE	LRT	d.f.	p-value	estimate	SE	LRT	d.f.	p-value
fixed effects										
twin	-0.901	0.231	14.754	1	**<0.001**	0.136	0.090	2.221	1	0.136
sex (male)	1.079	0.126	68.490	1	**<0.001**	0.384	0.062	37.414	1	**<0.001**
birth weight	2.486	0.182	258.81	1	**<0.001**					
age in August	0.072	0.011	40.359	1	**<0.001**	0.013	0.005	7.250	1	**0.007**
August FEC	-0.306	0.096	10.175	1	**0.001**					
August weight						-0.063	0.016	15.091	1	**<0.001**
maternal age	0.745	0.129				-0.001	0.060			
maternal age^2	-0.071	0.010	45.372	1	**<0.001**	<0.001	0.005	<0.001	1	0.987
April IgE	0.496	0.153	10.625	1	**0.001**					
August IgA						-0.272	0.122	4.900	1	**0.027**
random effects										
maternal ID	1.043					0.004				
year	0.614					0.085				
residual	1.207					0.381				
dropped fixed effects										
April IgE								0.053	1	0.818
April IgA			2.602	1	0.107			0.247	1	0.619
August IgE			0.374	1	0.541			2.074	1	0.150
August IgA			0.020	1	0.888					

Table 4.6. GLMM results of the final minimal model showing the effects of neonatal and August anti-*T. circumcincta* IgA and IgE levels on survival to August and first winter survival. Likelihood ratio test (LRT) results quoted for dropped fixed effects indicate these terms when singly added back to the final model. Significant results are shown in bold.

	Neonatal survival model					**Winter survival model**				
variables	estimate	SE	LRT	d.f.	p-value	estimate	SE	LRT	d.f.	p-value
fixed effects										
twin	1.095	0.543	4.138	1	**0.042**	0.009	0.394	<0.001	1	0.983
sex (male)	-0.357	0.364	0.971	1	0.325	-0.531	0.280	3.65	1	0.056
birth weight	2.638	0.550	27.193	1	**<0.001**					
August FEC						-0.339	0.198	2.927	1	0.087
August weight						0.180	0.072	6.452	1	**0.011**
maternal age	0.052	0.302				-0.398	0.265			
maternal age^2	-0.011	0.024	0.184	1	0.668	0.022	0.022	1.039	1	0.308
April IgE	1.977	0.619	13.329	1	**<0.001**					
dropped fixed effects										
April IgE								0.131	1	0.718
April IgA			1.134	1	0.287			0.016	1	0.898
August IgE								0.259	1	0.611
August IgA								0.364	1	0.546

4.5 Discussion

This is the first quantitative genetic study of maternal antibody transfer in a wild population and provides evidence that maternal genetic effects are important in influencing neonatal antibody levels. We have found important fitness consequences of variation in neonatal antibody levels for Soay lambs in terms of early growth and survival that are independent of other early life traits that are frequently measured. Although our results are correlational, and the mechanisms responsible for associations between anti-*T. circumcincta* antibody levels and growth and survival remain undetermined, this study provides rare evidence for a fitness benefit of maternally transferred antibodies in the wild.

4.5.1 Predictors of neonatal (April) antibody levels

Capture age and birth weight of the lamb, as well as maternal age, were important predictors of neonatal anti-*T. circumcincta* IgA and IgE levels. The strong decline of neonatal antibody levels with capture age of Soay lambs has been documented in pigs and ruminants (Thatcher & Gershwin 1989; Argüello *et al.* 2004; Hernández-Castellano *et al.* 2015), and is associated with the closure of the gut to antibody absorption at around 24 hours and subsequent clearance of antibodies from the bloodstream (Brambell 1970; Butler 1999). Neonatal antibody levels were also associated with birth weight of the lamb. The presence of higher neonatal antibody levels in heavier born offspring has also been documented in goats (Castro *et al.* 2009) and may be due to increased suckling ability or due to good quality mothers producing both heavier lambs and good quality colostrum. In addition, lighter lambs may have been born prematurely, a factor which reduces protein uptake ability in pigs and ruminants (Sangild 2003). We also found a curvilinear association of maternal antibodies with maternal age, with lower levels observed in lambs from the youngest and oldest mothers. However, the inclusion of birth weight, which shows a similar relationship with maternal age (Hayward *et al.* 2013), in our IgA model made the association with maternal age negative and linear. There is clear evidence of an increase in colostrum IgG in domestic cows with parity or age (Kruse 1970; Muller & Ellinger 1981; Conneely *et al.* 2013), although a study in goats found no effect of parity on colostrum IgG levels (Argüello *et al.* 2006). It is likely that we are picking up a sign of senescence in maternal provisioning in Soay sheep, which has been observed previously with a curvilinear relationship of maternal

age on lamb birth weight (Hayward *et al.* 2013) that would not be possible to detect in domestic animals since they are culled before old age. In addition, this curvilinear association may be driven by improved immunity in middle-aged ewes, which is also the age group with the lowest strongyle FEC levels (Hayward *et al.* 2009). Unexpectedly, we also found twins had higher neonatal IgA levels after accounting for birth weight, but this is supported by evidence of increased colostrum IgG content with increased litter size (Gilbert *et al.* 1988) and may be due to heavier ewes in better condition being more likely to have twins (Clutton-Brock & Pemberton 2004) . The associations of birth weight and maternal age with neonatal antibody levels in Soay lambs suggest that maternal investment in offspring through provisioning of maternal antibodies may be closely linked to the condition of the mother.

To our knowledge, this study is the first to use a quantitative genetic approach to decompose variation in neonatal antibody levels into maternal genetic, maternal environment and additive genetic components in a wild mammal population. Maternal effects clearly represent an important source of variance in neonatal anti-*T. circumcincta* antibody levels and there is strong evidence that a substantial proportion of these maternal effects are genetic in origin. In addition, genetic differences between lambs explain about half of the variance explained by genetic differences between mothers. A genetic basis for maternal antibody transfer has been documented in the consistency of colostrum IgG content in domestic animals (Dardillat *et al.* 1978; Norman *et al.* 1981), as well as genetic markers of neonatal IgG levels (Laegreid *et al.* 2002; Clawson *et al.* 2004; Rohrer *et al.* 2014). A quantitative genetic study of serum immunoglobulin levels in piglets also found that the variance explained by maternal genetic effects was four times larger than the additive genetic component (Rohrer *et al.* 2014). Strong maternal effects, as well as maternal genetic effects, have also been documented for other early life traits in the Soay sheep including birth weight, birth date and litter size (Wilson *et al.* 2005). The presence of a maternal genetic component represents a heritable source of variance where the impact on the evolutionary response to natural selection is, in part, dependent on the covariance between additive genetic and maternal genetic effects (Kirkpatrick & Lande 1989; Wolf *et al.* 1998). In line with this, a number of selection studies have provided evidence that maternal performance markers, such as quantity or quality of milk, appear to be negatively correlated with offspring growth. For instance, calves from lines selected for elevated weaning or yearling weight had lower maternally derived IgG1 levels (Bradley, Niilo & Dorward 1979; Muggli

et al. 1984). In our study, we found no significant direct additive genetic variance underlying neonatal IgA levels and therefore we could only estimate the direct-maternal genetic correlation for neonatal IgE, which was not significantly different from zero. The large standard errors around our estimates of this correlation indicate a lack of power, and a larger dataset is needed to provide estimates with greater confidence. Despite these shortfalls, it is clear that neonatal anti-*T. circumcincta* antibody levels are largely controlled by maternal effects, particularly genetic differences between mothers, rather than genetic differences between lambs themselves.

4.5.2 Predictors of August antibody levels

On average, antibody levels declined from April to August suggesting that levels of antibodies that are maternally transferred cannot be sustained endogenously by the lamb's developing immune system until the subsequent sampling point around four months later. There was a tendency for lambs with high antibody levels of one isotype in August to have high antibody levels of the other, suggesting a coordination between production of IgA and IgE within individuals. Compared to the neonatal antibody models, we found fewer significant predictors of August antibody levels. In both antibody isotypes, we found that there was a positive association of antibodies with the lamb's age, and males appeared to have lower antibodies. The association with age may reflect the development of immunity in lambs, a previous study documented a two-fold increase in anti-*T. circumcincta* IgA levels from lambs to yearlings in the Soay sheep (Coltman *et al.* 2001b) and in Chapter 2 we noted a large increase in anti-*T. circumcincta* IgA, IgE and IgG between lambs and yearlings. It is well known that males often harbour higher parasite burdens and lower immune responses compared with females, a factor that has been attributed to either ecological or physiological (usually testosterone) causes (Zuk & Stoehr 2002). In support of this, we found that males had higher strongyle FEC burdens and lower antibody levels at 4 months old.

We observed a reduction in the proportion of variance in antibody levels explained by maternal effects from 36-37% to 16-12% between neonatal and August antibody measures. There was also an increase in the proportion of variance in antibody levels explained by additive genetic variance from 9-14% to 28-15% between these two measures. In addition, we saw an increase in residual variance, suggesting more unknown or unmeasured sources of

environmental variance in older lambs. This is likely due to neonatal antibody measures being directly influenced by the colostrum content which is largely determined by the mother's genes, while later measurements are a result of the lamb's endogenous antibody production which are likely influenced by complex interactions between exposure, condition and genetic predisposition. The total proportion of variance in August antibody levels explained by all random effects was markedly lower for IgE than IgA, explaining only 29% of the variance in IgE levels compared to 49% of the variance in IgA levels. This may be due to that fact that IgE antibodies have a short half-life and spend very little time in the circulation before being bound to mast cells, basophils or eosinophils (Murphy 2012). Previous studies of domestic lambs have found that genetic variation among lambs has an important influence on parasite-specific antibody levels. Heritability estimates of anti-*T. circumcincta* IgE were 0.39±0.16 against third stage larvae and 0.50±0.16 against fourth stage larvae (Murphy *et al.* 2010) while heritability of anti-*T. circumcincta* IgA was 0.56±0.11 against fourth stage larvae (Strain *et al.* 2002). In Chapter 2, we also found heritabilities of 0.45±0.05 for IgA and 0.15±0.03 for IgE in lambs which are similar to the estimates using the current smaller dataset (IgA 0.29±0.12; IgE 0.15±0.11) although the former are more robust due to the much larger dataset used. An interesting next step would be to run bivariate models to investigate the covariance and correlation between maternal effects associated with antibody levels in neonates and lambs. If the maternal effects are independent and uncorrelated it would imply that different aspects of the maternal phenotype are influencing antibody levels at each time point and that selection could act on them independently. However, the bivariate models were underpowered and require a much greater sample size to estimate with confidence the covariances and correlations that we are interested in.

4.5.3 Consequences of early life immunity

There appear to be important fitness consequences of variation in neonatal antibody levels in Soay sheep as documented by significant positive associations with growth and survival. In particular, lambs that had high neonatal antibody levels were more likely to survive to, and were heavier at, four months of age. Maternally transferred antibodies have been associated with improved growth rate of offspring and this has been observed in birds (Buechler *et al.* 2002; Grindstaff 2008), domestic livestock (Robison *et al.* 1988) and laboratory mice (Gustafsson *et al.* 1994). This benefit may be due to a trade-off of resources between growth

and immunity, since neonates with sufficient maternal antibodies are able to clear pathogens without having to induce a costly innate immune response (Grindstaff 2008). Maternal antibodies may also benefit growth indirectly, by non-specifically stimulating cell surface receptors involved in growth (Gustafsson *et al.* 1994) or may be correlated with nutrient quality provided by the mother (Massimini *et al.* 2006). Maternal antibodies have also been documented to enhance survival during the early part of life (Gilbert *et al.* 1988; Gustafsson *et al.* 1994) which may be due to the direct protection provided by maternal antibodies against micro- and macroparasites (Dohmae *et al.* 1993; Kallio *et al.* 2006). However, we did not see any association of neonatal antibodies with first winter survival, suggesting benefits of maternal antibodies are relatively short-lived, as expected from their strong decline with capture age, but more data are needed to confirm this conclusively.

Despite the short period of protection offered by maternal antibodies, as documented by the strong decline with capture age in our neonatal data, maternal antibodies can have longer term effects on the development of the offspring's endogenous immunity. Some studies have proposed a beneficial effect of maternal antibodies on the development of the offspring's humoral immunity. For instance, in mice, offspring produce higher antibody titres to antigens to which their mother has been exposed (Stern 1976; Okamoto *et al.* 1989) and this enhanced effect can even be detected in the F2 generation (Lemke, Lange & Berek 1994; Lundin *et al.* 1999). Such benefits have been potentially linked to maternal antibodies operating as templates and fulfilling an instructive role in the development of the humoral immune response (Anderson 1995; Lemke, Coutinho & Lange 2004). However, maternal antibodies have also been found to suppress the humoral immunity of offspring. A number of studies of vaccinations in human infants have shown that the presence of high maternal antibodies resulted in lower endogenous humoral responses to the vaccine (Albrecht *et al.* 1977; Björkholm *et al.* 1995; Troisi *et al.* 1997). Studies have typically found that B cell, but not T cell, responses are suppressed by maternal antibodies (Siegrist 2003). Selective suppression of B cell responses may be due to epitope blocking by maternal antibodies or clearance of maternal antibody coated antigens by Fc-dependent phagocytosis resulting in reduced levels of epitopes or antigens visible to infant B cells (Jelonek *et al.* 1996; Nohynek *et al.* 1999; Heyman 2001; Siegrist 2003). We found no clear evidence that maternal antibodies were associated, either positively or negatively, with endogenous antibody production at 4 months old. In support of the lack of influence on later anti-helminth immunity, there was no association of maternal antibodies on strongyle FEC at 4 months old.

Instead we found a weak negative association between August strongyle FEC and August IgA, suggesting that endogenous antibody production may be more important for parasite protection at this time point. A negative association between anti-*T. circumcincta* IgA and FEC is expected from the veterinary literature, which suggests that a protective effect of parasite specific IgA develops first in lambs and is important in reducing female worm length and fecundity (Stear *et al.* 1995, 1996; Strain *et al.* 2002). In Chapter 3, we documented that high IgA was associated with lower FEC in lambs, while high IgG was associated with lower FEC in adults. A larger study, additionally measuring anti-*T. circumcincta* IgG levels and a measure of total protein transfer, is needed to determine whether neonatal antibody levels are providing direct protection against parasites or may be blocking or priming the endogenous immune response.

4.5.4 Immune protection or maternal quality?

Determining whether the positive associations of neonatal antibodies on early growth and survival are due to actual transfer of immune protection in the form of anti-helminth antibodies is difficult in correlational studies. For instance, better resourced mothers may lead to offspring that have higher antibody levels with improved growth and survival, even if there is no causal relationship between antibody levels and offspring fitness. Alternatively, associations between neonatal antibodies and fitness would also be noted if they were simply measuring general quality or quantity of colostrum ingested and were reflecting a positive association between early-life nutrition and fitness. In this study we are limited to measuring antibody levels in the lamb: we are unable to measure antibody levels in the mother's plasma or colostrum since capturing the mother to sample blood and milk would cause excessive disturbance just around birth. A measure of the quality or quantity of colostrum ingested could help elucidate mechanisms at work. In the veterinary literature, colostrum total IgG is often used to separate high quality from low quality colostrum, and often a threshold concentration of total IgG in calf serum is set to indicate failure or success of passive transfer of immunity (Weaver *et al.* 2000; Bielmann *et al.* 2010). Measurement of total IgG may help to elucidate whether we are measuring anti-helminth protection since not all antibodies are effective and more are not necessarily better (Viney *et al.* 2005). Other markers of colostrum intake, such as serum gamma glutamyl transferase or serum total protein concentration, can also be used as markers of passive immune transfer in lambs and are correlated with total IgG in lamb serum (Maden *et al.* 2003; Britti *et al.* 2005; Massimini *et al.* 2006).

4.5.5 Further work

In this study we have documented an important early life trait that has important consequences on lamb fitness and survival, and this has opened up many future lines of investigation that could be explored with a larger dataset. We found an association of maternal age on neonatal antibody levels and it is very likely that other factors associated with maternal condition such as weight, parasite burden and environmental stressors, such as population density, may also affect the ability of ewes to invest in their offspring. It would also be interesting to investigate whether the levels of antibodies passed on to offspring are correlated with the mother's plasma parasite-specific antibody levels. In addition, there is a lack of information on costs to the mother in terms of transferring antibodies. Although it is expected that maternal antibodies would have a greater effect on offspring than mothers, there may still be a cost to mothers of investing in their offspring (Grindstaff *et al.* 2003). This may indicate an area of parent-offspring conflict in which offspring may benefit from more antibodies while mothers who transfer high antibody levels to offspring may suffer a drop in maternal condition and reduced investment in subsequent offspring (Boulinier & Staszewski 2008). Mothers may thus experience trade-offs relative to their potential investment in the transfer of maternal antibodies and it would be interesting to see the effects of variation in maternal antibody transfer on the mother, in terms of weight and strongyle burden, as well as survival and breeding success the following year.

Chapter 5

The causes and consequences of variation in maternally-transferred parasite-specific antibody levels for offspring and mothers

5.1 Summary

While studies in wild systems have documented benefits of maternally transferred antibodies for offspring growth and survival, it is unclear whether benefits are due to transfer of immunity to pathogens or due to general maternal provisioning. Furthermore, no studies (except for the preceding chapter) have investigated whether variation in maternally transferred antibody levels has a genetic basis, at the maternal and offspring level, and has linked these to health and fitness consequences for both offspring and mothers. Here, following on from the pilot study in Chapter 4, we measured neonatal antibody levels in wild Soay sheep in a much larger 25 year dataset in order to determine the causes and consequences of variation in maternally-transferred antibody levels for offspring and mothers. In addition to measuring IgA, IgE and IgG levels to a common nematode (*Teladorsagia circumcincta*), we measured total IgG levels as a proxy for the total amount of antibodies and general colostrum quality received by the neonate. Neonatal anti-*T. circumcincta* antibody levels were negatively associated with capture age, and positively associated with birth weight. We also found that prime-aged mothers, as well as mothers with high levels of antibodies in the previous year, had offspring with higher neonatal antibody levels. Quantitative genetic analyses revealed that there was no additive genetic variance underlying neonatal anti-*T. circumcincta* antibody levels, but maternal and maternal genetic effects explained a considerable proportion of the variance in these traits. There was also evidence for associations between neonatal anti-*T. circumcincta* antibody levels and later offspring phenotype and fitness. We found that neonatal anti-*T. circumcincta* IgG levels

positively predicted survival to four months old, as well as weight in August, independent of total IgG levels and August endogenous antibody levels. In addition, anti-*T. circumcincta* IgG levels were associated with reduced strongyle faecal egg counts (FEC) in August, and were associated with improved survival over the first winter via effects on FEC. We found no evidence that maternal investment in offspring antibody levels had negative consequences for her subsequent weight or FEC, and were not able to satisfactorily test for costs via maternal survival or fecundity due to issues with model convergence. This study, together with Chapter 4, represents the first comprehensive decomposition of variance in maternally transferred antibody levels between maternal genetic and offspring additive genetic effects in the wild and suggests that maternally-transferred parasite-specific antibody levels are associated with transfer of immunity to parasites as well as improved offspring growth and survival.

5.2 Introduction

Maternally-transferred antibodies provide passive immunity to neonates during a critical period when their immune system is not yet fully developed (Brambell 1970). In addition to direct protection against parasites, maternal antibody transfer may also save precious resources urgently needed for offspring growth, and could result in long-term priming of the offspring's immune response (Demas & Nelson 2012). Although maternal antibodies have been widely acknowledged to be associated with offspring growth and survival in domestic ruminants (Robison *et al.* 1988; Wittum & Perino 1995), evidence of benefits for offspring in the wild are rarer and may be limited by availability of suitable immunological reagents or information about natural host-parasite systems (Boulinier & Staszewski 2008; Hasselquist *et al.* 2012). Furthermore, despite the important evolutionary and ecological consequences of variation in maternal antibody transfer, studies in the wild have yet to disentangle the maternal and offspring genetic contributions to this variation (except for the pilot analysis in Chapter 4) or simultaneously estimate the consequences for both offspring and maternal fitness (Grindstaff *et al.* 2003; Boulinier & Staszewski 2008; Hasselquist & Nilsson 2009).

In ruminants, there is no prenatal transfer of immunoglobulins from the mother to the foetus and consumption of colostrum has a fundamental role in passive immune transfer (Brambell 1970; Campbell *et al.* 1977). In addition, the non-specific pinocytotic absorption of

immunoglobulins from the intestine of neonatal ruminants diminishes rapidly and ceases by 24-36 hours after birth due to gut closure (Butler 1999). As neonatal ruminants are unable to synthesise their own immunoglobulins for several weeks, immunoglobulin levels in the blood are entirely of maternal origin (McRae *et al.* 2015). After gut closure, immunoglobulins continue to be found in milk and this provides intestinal immunity against enteric disease (Pastoret *et al.* 1998; Tizard 2012). In addition to providing passive immunity to neonates, colostrum and milk provide general nutrition needed for neonate growth and survival (Blum & Hammon 2000). In Chapter 4, we found positive associations between neonatal anti-*Teladorsagia circumcincta* IgA and IgE levels and early life survival and growth. However, this dataset was only a fraction of the neonatal blood samples that have been collected during the project. It was also unclear whether the beneficial associations of neonatal anti-parasite antibodies were due to their specificity or to the general levels of antibody or protein transferred. Furthermore, anti-*Teladorsagia circumcincta* IgG levels were not measured in these samples, which is important because IgG has the longest half-life of all maternally transferred antibody isotypes (Husband, Brandon & Lascelles 1972) and has also been associated with reduced strongyle FEC in adult Soay sheep, improved over-winter survival and higher fecundity in adult males (Chapter 3, Nussey *et al.* 2014; Watson *et al.* 2016).

In this chapter we measured anti-*T. circumcincta* antibody levels in all samples of neonatal Soay lambs caught during the lambing season over a 25 year period between 1990-2015 providing us with a much larger dataset with greater power compared to analyses in Chapter 4. In addition to measuring anti-*T. circumcincta* IgA and IgE levels as before, we also measured anti-*T. circumcincta* IgG and total IgG. In ruminant colostrum, IgG is the predominant immunoglobulin, accounting for between 65-90% of the total antibody content (Butler 1999; Tizard 2012) and failure of passive immune transfer is measured by total IgG levels in the serum of calves (Weaver *et al.* 2000; Gulliksen *et al.* 2008; Bielmann *et al.* 2010). Measuring total IgG levels provides a way of controlling for total antibody and protein transferred and would allow us to disentangle the beneficial effects of anti-parasite antibodies from the general quality of the colostrum (Demas & Nelson 2012). If the quality of colostrum and milk are consistent across lactation, this measure could tell us something about the quality of the milk received by the neonate across the entire lactation period (Butler 1999). The use of a greater scope of immune markers facilitates our understanding of

the beneficial effects of maternal antibodies on offspring growth and fitness seen in the wild, in relation to an ecologically relevant host-parasite interaction.

Immune responses are costly to produce and maintain, and therefore it is likely that females that transfer high levels of maternal antibodies may have an associated cost (Grindstaff *et al.* 2003; Boulinier & Staszewski 2008; Hasselquist & Nilsson 2009; Demas & Nelson 2012). Since levels of maternally transferred antibodies are generally associated with levels produced by the mother (Grindstaff *et al.* 2003), the transfer of high levels may be a resource drain due to the cost in eliciting and maintaining an immune response (Lochmiller & Deerenberg 2000; Schmid-Hempel 2003). In support of this, there is evidence that maternal condition and nutrition are positively associated with maternally transferred antibody levels (Morales, Sanz & Moreno 2006; Pihlaja *et al.* 2006; Hargitai, Prechl & Török 2006; Karell *et al.* 2008). In addition to the close association between maternal condition and maternal antibody transfer, loss of condition following transmission of passive immunity may lead to life-history trade-offs and a reduced ability to invest in next year's offspring (Demas & Nelson 2012). However, thus far, research has focussed on costs and benefits of maternal antibodies for offspring in wild systems and not their mothers (Hasselquist & Nilsson 2009). In this chapter we are able to test for maternal costs, which were not possible with the reduced dataset in Chapter 4.

The aim of this chapter is to investigate the predictors and consequences of maternally transferred antibody levels in a long-lived wild mammal in a much larger dataset which was collected following the pilot study in Chapter 4. Firstly, we tested for associations between offspring and maternal factors associated with variation in neonatal anti-*T. circumcincta* antibody levels and performed quantitative genetic analysis to partition the phenotypic variance in neonatal antibodies levels into additive genetic, maternal genetic and environmental sources of variation. Using data collected subsequently and reported in Chapter 2 and 3, we were also able to investigate whether maternally transferred levels were associated with the mother's plasma antibody levels measured in the preceding August. We then tested for associations between neonatal antibody levels and strongyle FEC levels, weight, and neonatal and first-winter survival. The availability of total IgG levels in our dataset also allowed us to investigate whether any health and fitness benefits linked to higher neonatal anti-*T. circumcincta* antibody levels found in Chapter 4 were independent of total

antibody levels and the quality of the colostrum. Finally, this larger dataset also enabled us to look at costs to the mother, and we did this by looking at associations with weight, parasite FEC and survival, in addition to breeding success in the following year. From the results in Chapter 4, we predict that neonatal antibody levels will be associated with offspring health and fitness benefits, but some of these associations may be due to general colostrum quality. In addition, we might expect there to be costs associated with the levels of total IgG transferred from mother to lamb.

5.3 Methods

5.3.1 Study population

Soay sheep are a primitive breed of domestic sheep that were isolated on the island of Soay in the remote St Kilda archipelago several millennia ago, and have been living under unmanaged conditions and evolving under natural selection since then (Clutton-Brock & Pemberton 2004). In 1932, just over 100 Soay sheep were moved to the larger island of Hirta after the evacuation of all human residents. Approximately a third of the current population of these sheep live in the Village Bay area of Hirta, and these individuals have been the subject of a long-term study since 1985 (Clutton-Brock & Pemberton 2004). In April each year around 95% of all lambs born in this area are caught and individually tagged. At capture during the lambing season, neonatal lambs are weighed and blood samples are collected. Each August, when lambs are around 4 months old, as many sheep as possible from the study population are re-captured using temporary traps (Clutton-Brock & Pemberton 2004). At capture in August, animals are weighed and blood and faecal samples are collected. At both time points, whole blood samples are collected into heparin tubes, centrifuged at 3000 r.p.m. for 10 minutes, and plasma removed and stored at -20^{0}C. August strongyle faecal egg count (FEC) is estimated from faecal samples as the number of eggs per gram using a modified McMaster technique (Gulland & Fox 1992). Contributing to the majority of the strongyle FEC are three species: *T. circumcincta, Trichostrongylus axei* and *Trichostrongylus vitrinus* (Craig *et al.* 2006). The majority of deaths occur over winter, and the population dynamics of the Soay sheep are characterised by periods of low but rising population sizes followed by high mortality 'crash' winters in which over half the population can die (Clutton-Brock & Pemberton 2004). Regular censuses and mortality searches during

the winter months results in the majority of carcasses being discovered and provides accurate death date information.

In this analysis we included all neonate lambs that were caught and subsequently had plasma samples taken in the lambing season (March-May) between 1990 and 2015. No April samples or data were collected in 2001 due to Foot and Mouth disease precautions. This comprised 3496 samples from 3491 individuals over the 25 year period. This included the plasma samples used in the pilot study in Chapter 4, however, for this chapter all samples across the 25 years were re-run in the lab for consistency. In addition, several improvements to the laboratory methods were carried out most notably: multiple years run across a plate (to prevent the confounding of plate and year) and the use of a corrected OD ratio (see below).

5.3.2 Laboratory methods

IgA, IgG and IgE activity against antigens of the third larval stage of *T. circumcincta* (henceforth, "anti-Tc antibodies") and total IgG levels were measured using direct (IgA, IgG) and indirect (IgE) ELISAs. We used *T. circumcincta* L3 somatic antigen, provided by the Moredun Research Institute, as the capture antigen for the *T. circumcincta* assays diluted to 2µg/ml in 0.06M Carbonate buffer at pH 9.6. For the total IgG assay, we diluted rabbit anti-sheep IgG (Bio-Rad 5184-2104) to 2µg/ml in 0.06M Carbonate buffer at pH 9.6. 50µl of the diluted capture antibody/antigen was added to each well of a Nunc-immuno 96-microwell plate, which was covered and incubated at 4^{o}C overnight. After washing the wells three times in Tris-buffered saline-Tween (TBST) using a plate washer, 50µl of the Soay sheep plasma sample diluted to 1:50 for anti- *T. circumcincta* IgA and IgE, 1:12800 for anti-*T. circumcincta* IgG and 1:819200 for total IgG was added to each well. The plates were then covered and incubated at 37^{0}C for 1 hour. Plates were then washed five times with TBST and 50µl per well of rabbit anti-sheep IgA detection antibody conjugated to horseradish peroxidase (HRP) (AbD Serotec AHP949P) diluted 1:16000 was added to the anti-*T. circumcincta* IgA assay and 50µl per well of rabbit anti-sheep IgG detection antibody conjugated to HRP (AbD Serotec 5184-2104) diluted 1:16000 was added to the anti-*T. circumcincta* IgG and total IgG assays. For the anti-*T. circumcincta* IgE assay, 50µl per well of anti-sheep IgE (mouse monoclonal IgG1, clone 2F1, provided by the Moredun Research Institute) diluted 1:100 was added, followed by 1 hour incubation at 37^{0}C, five washes with TBST and then 50µl per well of goat anti-mouse IgG1-HRP detection antibody (AbD

Serotec STAR132P) diluted to 1:8000 in TBST was added. All plates were then incubated at 37^0C for 1 hour. Plates were then washed five times with TBST and 100µl of SureBlue TMB 1-Component microwell peroxidase substrate (KPL) was added per well and left to incubate for 5 minutes in the dark at 37^0C. Reactions were stopped by adding 100µl per well of 1M hydrochloric acid and optical densities (OD) were read immediately at 450nm using a Thermo Scientific GO Spectrophotometer.

All results were recorded as OD values. In order to minimise confounding capture year effects with plate to plate variation, each plate included samples from two years paired at random. All plates were run in duplicate and duplicate sample ODs were removed if the coefficient of variation was > 0.2 and the difference between ODs was greater than 0.2. We also checked the correlation of ODs across duplicate plates and re-ran both plates if $r < 0.8$. To reduce error due to within-plate variation, per plate we included two sample free wells (50µl TBST) as blanks and two wells of positive controls. Positive controls for the IgE assay were serum from ewes trickle infected with *T. circumcincta* and for the IgA and IgG assay were plasma from normal healthy non-immunised domestic sheep. For subsequent analyses, the mean optical density ratio of each sample was taken according to this formula:

$$OD = \frac{(\text{sample OD} - \text{blank OD})}{(\text{positive control OD} - \text{blank OD})}$$

Where the numerator was set to zero if the blank OD was greater than the sample OD in order to avoid negative values. Distributions of antibodies are shown in Figure 5.1. The number of samples that failed quality control per assay was 16 for anti-*T. circumcincta* IgA, 11 for anti-*T. circumcincta* IgE, 17 for anti- *T. circumcincta* IgG, and 15 for total IgG.

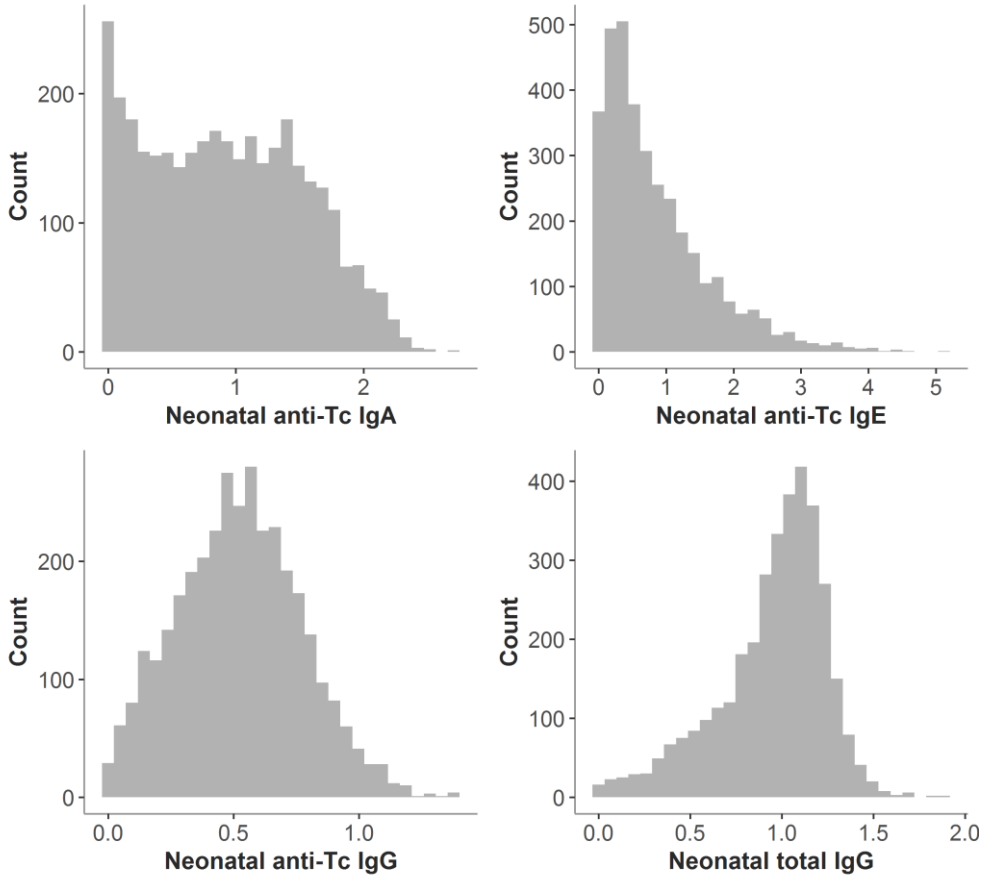

Figure 5.1. Histograms of anti-*Teladorsagia circumcincta* IgA, IgE, IgG and total IgG levels in neonatal Soay lambs.

5.3.3 Statistical analyses

Five individuals had two samples, and these duplicates were removed, taking the first measurement if samples were collected on different days, or picking one of the duplicates at random if not. One lamb born late in the season (July) was removed from the dataset. For the analysis we reduced the dataset to all animals caught within 10 days of birth for three reasons. Firstly, in order to accurately estimate birth weight from capture weight since the relationship between capture age and capture weight changes dramatically after this time point. Secondly due to a sharp decline of antibodies in older lambs (Figure 5.2) and finally to account for low sample sizes in older capture age groups (n = 85). In total we had a sample size of 3379 lambs from 845 mothers and the dataset contained 52% females and 24% twins.

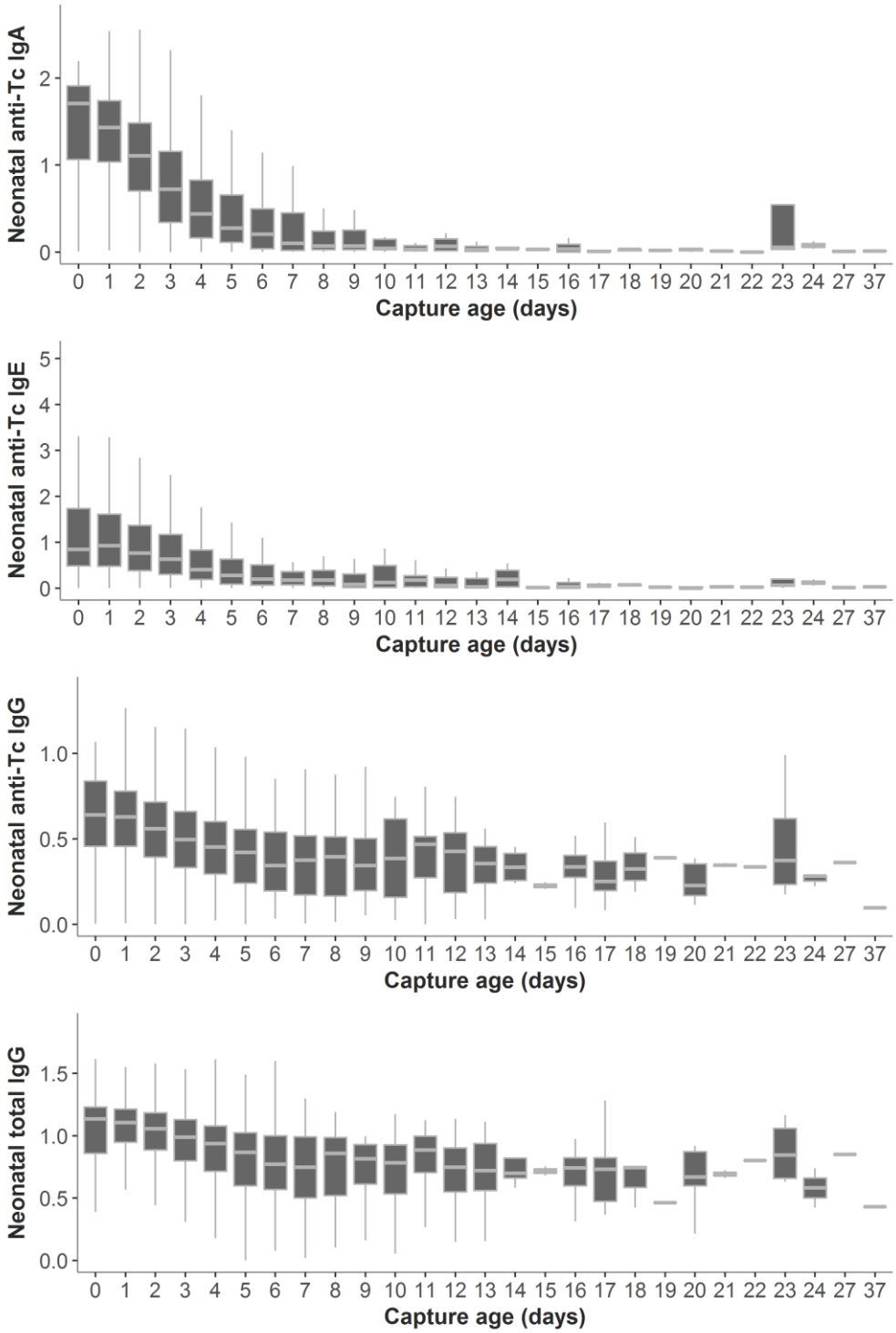

Figure 5.2. Boxplots of raw data showing associations between neonatal antibody levels and capture age in days since birth for anti-*Teladorsagia circumcincta* IgA, IgE, IgE and total IgG in wild Soay sheep. Boxes show the median and the interquartile range (IQR) with whiskers extending from the hinges to values no further than 1.5*IQR from the hinge (outliers not shown).

Models of antibody levels

We examined potential causes of variation in neonatal anti-Tc IgA, IgE and IgG levels using linear mixed effects models with antibody levels as a response variable in the "lme4" package in R 3.3.3. These models included maternal identity, year, plate and run date as random effects to account for variation among maternal siblings, years and experimental variation. We first fitted a model containing fixed effects selected as offspring and maternal traits that had previously been identified as important predictors of lamb state and fitness (Clutton-Brock *et al.* 1992, 1996; Hayward *et al.* 2013). We then simplified the model by step-wise deletion, and sequentially removed fixed effects with the lowest non-significant t values and determined statistical significance using likelihood ratio tests until a base model containing only significant ($p < 0.05$) fixed effects was left. All dropped non-significant terms were then re-tested against this base model using the same criteria.

For the neonatal antibody models we included lamb sex, twin status, residual birth weight, capture age of the lamb, and maternal age (linear and quadratic terms) as fixed effects. A variety of functions of capture age were fitted to the models to determine which best explained variation in neonatal antibody levels. Linear and quadratic functions of capture age were compared with threshold models with a single threshold at day 1 to 9 (following Berman, Gaillard, and Weimerskirch 2009). The best model of capture age was chosen based on the lowest AIC value, unless the difference between the two best fitting models was < 2 AIC values, in which case the most parsimonious model was chosen. Since Soay lambs are not caught at birth, birth weight was estimated by taking the residuals from a linear model with a quadratic function of capture age on capture weight. Birth weight was included in analyses to identify factors that were associated with neonatal antibody levels independent of birth weight, as it could be that these traits are correlated if better resourced mothers were able to produce both heavier lambs at birth and high quality immunoglobulin-rich colostrum.

Animal model

We fitted quantitative genetic "animal models" in order to determine the maternal genetic and additive genetic variance underlying anti-Tc antibody levels in ASReml-R 3.0 (Butler *et al.* 2009). The pedigree used was constructed using maternities and paternities assigned with 315 unlinked single nucleotide polymorphisms (linkage disequilibrium $r^2 < 0.05$) with a minor allele frequency > 0.4 using the R library *sequoia* (Huisman 2017). This pedigree included all cohorts from 1985-2015 and includes 8221 individuals with 7142 maternities and 5456 paternities.

Univariate animal models were fitted for each of the three anti-*T. circumcincta* antibody measures. Fixed effects were included as determined from the LMM analyses, but excluding birth weight due to potential genetic correlations between these traits (Wilson 2008). The random effects included the additive genetic component, maternal genetic and environment components, birth year, ELISA plate number and run date of the ELISA. Significance of random effects was determined by dropping each random effect from a model containing all random effects and performing a likelihood ratio test on the two log likelihoods.

The proportion of the phenotypic variance explained by each random effect was estimated as the ratio of the relevant variance component to total phenotypic variance as defined as the sum of all variance components. The heritability of each measure was determined as the ratio of the additive genetic variance to the total phenotypic variance. Standard errors of ratio components were calculated using the pin function from R package "nadiv" (Wolak 2012).

Models of offspring growth, parasite FEC and survival

1823 of the 3379 lambs (54%) caught in April were caught again at around four months old in August. We built models with neonate survival (survival to 1st August), weight and strongyle FEC at August capture, and first winter survival as response variables and tested whether there were any associations with neonatal anti-Tc antibody levels. Due to the strong association between neonatal anti-Tc antibodies and capture age, neonatal anti-Tc IgA, IgG and IgE levels were corrected for capture age by taking residuals from a model with a threshold at day 4 for IgA and IgE and day 7 for IgG (see results). To each of the minimal models (see below), we added each neonatal anti-Tc antibody isotype separately and tested the significance of these terms using likelihood ratio tests. To test whether these associations were due to the total amount of antibodies, and presumably maternal protein transfer more generally, in the colostrum, rather than to nematode parasite-specific antibodies, we next tested whether neonatal anti-Tc antibodies were significant with neonatal total IgG in the model. Neonatal total IgG was corrected for capture age by taking residuals from a model with a linear effect of capture age on total IgG levels. Finally, we checked whether the neonatal anti-Tc antibodies were significant with both neonatal total IgG and the lamb's endogenous antibody levels of the same isotype measured in August in the model.

We investigated potential associations between August weight and anti-*T. circumcincta* antibody levels using linear mixed effects models via the "lme4" package, assuming a Gaussian distribution. 1818 of 3379 lambs caught in April had August weight information. We included the fixed effects determined in Chapter 2, as sex, twin status, birth weight and age in days, in addition to maternal age (linear and quadratic terms) due to the association with neonatal antibody levels. The random effects included in the model were capture year and maternal identity.

August strongyle FEC was heavily skewed and zero-inflated. In order to better approximate a Poisson or binomial distribution, we binned August strongyle faecal egg count (FEC) into multiples of 100, with 0s counted as 0, and 4 lambs with > 4,000 eggs grouped at a value of 41. 1621 of 3379 lambs caught in April had strongyle FEC data in August. Associations between neonatal antibody measures and August FEC was modelled using generalised linear mixed models (GLMMs) via the "glmmADMB" package fitting FEC as the explanatory variable with a negative binomial "NB2" distribution without zero-inflation following Chapter 3. We included twin status, sex and weight (linear and quadratic) as fixed effects (as in Chapter 3), in addition to maternal age (linear and quadratic terms) due to the association with neonatal antibody levels. The random effects included in the model were capture year and maternal identity.

We calculated neonate survival and first winter survival as sheep that survived to 1st August of that year and 1st May in the subsequent year respectively, using death date information, capture and census information. All 3379 neonatal lambs had neonatal survival information, while 3064 had first winter survival information. Only 5% of lambs did not survive to August, while 57% did not survive their first winter. Such low levels of neonatal deaths in the dataset, despite high neonatal mortality in general, is likely due to lack of blood samples taken from lambs that die close to birth (within 2 days) which is likely to cause a loss in the proportion of neonatal mortality. Analyses of survival were performed using GLMMs in the package "lme4" with a binomial error structure. Binomial GLMMs were run with the built-in "bobyqa" optimiser and the maximum number of iterations increased to $2e^{-6}$ to improve model convergence. Continuous variables were rescaled to mean 0 and standard deviation 1 prior to inclusion in each model subset. For the neonatal survival model we included sex, twin status, birth weight and maternal age as fixed effects, and birth year and maternal ID as

random effects. For the first winter survival model we included sex, weight and a sex by weight interaction as fixed effects and year as a random effect (as in Chapter 3).

Models of maternal growth, parasite FEC, survival and breeding success

To determine whether there was evidence for costs to mothers of maternal antibody transfer we looked at associations between neonatal antibody levels and maternal weight, FEC, survival and breeding success. After determining the fixed effects as stated below, we added neonatal anti-Tc antibodies and total IgG antibody levels separately and tested the significance of these terms using likelihood ratio tests. To test whether any significant associations with offspring neonatal anti-Tc antibodies were independent of total offspring protein transfer, we next tested whether neonatal anti-Tc antibodies were significant with neonatal total IgG included in the model. Finally, we checked whether the neonatal anti-Tc antibodies were significant with both neonatal total IgG and the mother's endogenous antibody levels of the same isotype measured in August in the model.

We investigated potential associations between August weight and maternally transferred antibody levels using linear mixed effects models via the "lme4" package, assuming a Gaussian distribution. We ran the model twice, one with and the other without mother's weight last year as a fixed effect in the model, in order to look at weight change since the previous year, which includes the period of lambing. 439 mothers with 1152 lambs had August weight data for both the current and previous year. We also included the mother's age (as linear and quadratic terms) as well as characteristics of the lamb (sex, twin status and corrected birth weight) in the fixed effects and included the mother's identity and year as random effects. We dropped any non-significant fixed effects from the model based on likelihood ratio tests.

August strongyle FEC was heavily skewed and zero-inflated. In order to better approximate a Poisson or binomial distribution, we binned August strongyle faecal egg count (FEC) into multiples of 100, with 0s counted as 0, and 2 mothers with > 13,000 eggs grouped at a value of 13. We ran the model twice, one with and the other without mother's strongyle FEC last year (also binned) as a fixed effect in the model, in order to look at FEC change since the previous year, which includes the period of lambing. 400 mothers with 967 lambs had

August FEC data for both the current and previous year. Associations between neonatal antibody measures and August FEC was modelled using GLMMs via the "glmmADMB" package fitting FEC as the explanatory variable with a negative binomial "NB2" distribution without zero-inflation following Chapter 3. We also included the mother's age (as linear and quadratic terms) as well as characteristics of the lamb (sex, twin status and corrected birth weight) in the fixed effects and included the mother's identity and year as random effects. We dropped any non-significant fixed effects from the model based on likelihood ratio tests.

We defined winter survival for mothers as survival to 1st May in the year following lambing and calculated it using death date information, capture and census information. 812 mothers of 3252 lambs had subsequent winter survival information, of which 16% did not survive. Analyses of survival were performed using GLMMs with a binomial error structure in the package "lme4". Binomial GLMMs were run with the built-in "bobyqa" optimiser and the maximum number of iterations increased to $2e^{-6}$ to improve model convergence. Continuous variables were rescaled to mean 0 and standard deviation 1 prior to inclusion in each model subset. We included the mother's age (linear and quadratic terms), the mother's weight (linear and quadratic terms) as well as characteristics of the lamb (sex, twin status and corrected birth weight) in the fixed effects and year and maternal identity as random effects. We dropped any non-significant fixed effects from the model based on likelihood ratio tests.

For the mother's annual breeding success, we used the number of offspring born to the ewe in the subsequent spring from observational data, excluding all individuals that did not survive the winter. 731 mothers of 2725 lambs had annual breeding success information next year, with 94% having a lamb in the subsequent year. Annual breeding success was treated as a binary measure and was run as a GLMM with a binomial error structure. We included the mother's age and weight as fixed effects, in addition to corrected birth weight of the lamb if significant. Maternal identity and year were included as random effects. We dropped any non-significant fixed effects from the model based on likelihood ratio tests.

5.4 Results

5.4.1 Predictors of neonatal antibody levels

There were weak to moderate positive correlations between antibody isotypes, but a strong correlation between neonatal anti-Tc IgG and total IgG levels (Figure 5.3). Capture age, birth weight, twin status and sex were associated with neonatal anti-Tc IgA, IgE and IgG levels (Table 5.1). The best fitting function of capture age on neonatal antibody levels were best defined by a threshold model with an inflection point at day 4 for IgA and IgE, and day 7 for IgG (ΔAIC to next best model for IgA = -16.345, IgE = -0.663, IgG = -0.378). For IgA and IgE, neonatal antibody levels declined linearly with age at capture, and this decline was steepest between 0-4 days old (Table 5.1, Figure 5.4). There was also a linear decline in anti-Tc IgG levels with capture age up to 7 days after which there was no significant linear relationship with capture age (Table 5.1, Figure 5.4). Birth weight was also associated with neonatal anti-Tc IgA, IgE and IgG levels, with heavier born lambs having higher antibody levels of all isotypes (Table 5.1, Figure 5.4). Twins were more likely to have higher neonatal antibody levels of all three isotypes, while males tended to have lower anti-Tc IgG levels (Table 5.1, Figure 5.4).

Maternal age, as well as the mother's anti-Tc antibody levels the previous August, were also predictive of neonatal antibody levels. There was a curvilinear association of maternal age with anti-Tc IgE and IgG levels, in which offspring of prime-aged mothers had higher antibody levels (Table 5.1, Figure 5.5). In contrast, anti-Tc IgA levels decreased with maternal age, but a similar curvilinear association was seen if birth weight was dropped from the model (without birth weight in the model: maternal age^2 - $\chi^2_{(1)} = 13.407$, $p < 0.001$). Neonatal antibody levels were also associated with maternal antibody levels of the same isotype measured in the preceding August (Table 5.1, Figure 5.5). The exception was neonatal anti-Tc IgG levels which were predicted by both previous maternal anti-Tc IgE and IgG levels. However, when both previous maternal anti-Tc IgE and IgG were fitted in the same model, only anti-Tc IgG levels were significant (Table 5.1; IgE: $\chi^2_{(1)} = 7.127$, $p = 0.008$, IgG: $\chi^2_{(1)} = 35.036$, $p < 0.001$).

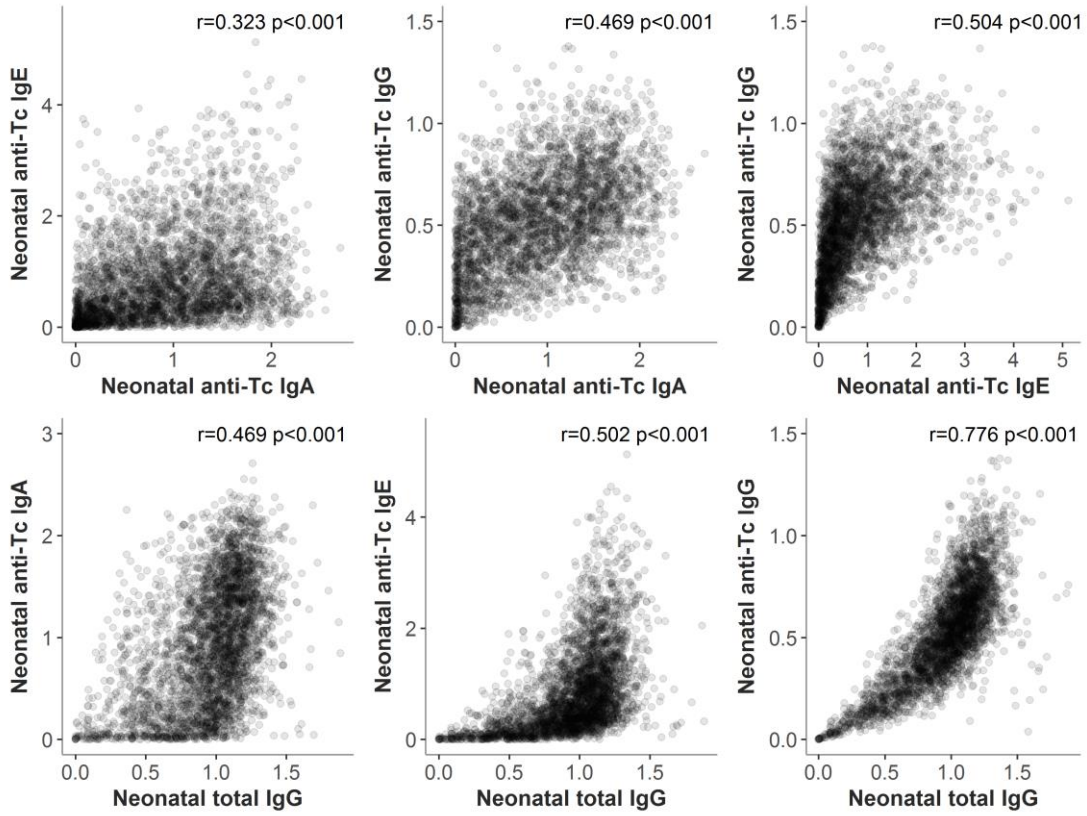

Figure 5.3. Scatterplots of raw data showing correlations between neonatal anti-*T. circumcincta* IgA, IgE, IgG and total IgG antibody levels in Soay sheep lambs. Correlation coefficients (r) and p-values quoted are from Pearson correlation tests.

Table 5.1. LMM results of the final minimal model for neonatal anti-*T. circumcincta* IgA, IgE and IgG levels in St Kilda Soay sheep. Included are the estimated effects (estimate), standard error (SE) and the significance of fixed effects based on a likelihood ratio test (LRT, d.f., p-value). Dropped fixed effects show the significance of adding dropped terms back to the minimal model. For the added fixed effects, each of the mother's antibody measures taken in the August preceding was added separately to the minimal model. Where the quadratic maternal age terms are significant, estimated effects are stated for both the linear and quadratic terms in together. t indicates a threshold model that best fits the capture age relationship, in IgA and IgE models this is at day 4 and in the IgG model this is at day 7.

	Neonatal anti-Tc IgA levels					**Neonatal anti-Tc IgE levels**					**Neonatal anti-Tc IgG levels**				
variables	estimate	SE	LRT	d.f.	p-value	estimate	SE	LRT	d.f.	p-value	Estimate	SE	LRT	d.f.	p-value
	n=3213					n=3218					n=3212				
fixed effects															
intercept	1.863	0.037				0.653	0.069				0.541	0.026			
twin	0.129	0.023	31.014	1	<0.001	0.075	0.031	5.828	1	0.016	0.044	0.012	13.185	1	<0.001
sex (male)											-0.024	0.006	13.894	1	<0.001
capture age: ≤t days	-0.289	0.008	1033.300	1	<0.001	-0.154	0.010	224.630	1	<0.001	-0.043	0.003	238.740	1	<0.001
capture age: >t days	-0.068	0.008	63.638	1	<0.001	-0.073	0.010	50.764	1	<0.001	0.013	0.011	1.289	1	0.256
birth weight	0.098	0.017	32.006	1	<0.001	0.198	0.024	68.095	1	<0.001	0.138	0.009	209.390	1	<0.001
maternal age	-0.044	0.004	153.940	1	<0.001	0.236	0.018	-	-	-	0.052	0.007	-	-	-
maternal age^2						-0.020	0.001	184.93	1	<0.001	-0.006	0.001	93.212	1	<0.001
dropped fixed effects															
sex			0.754	1	0.385			0.749	1	0.387					
maternal age^2			1.259	1	0.262										
added fixed effects															
maternal IgA	0.310	0.024	141.750	1	<0.001			1.846	1	0.174			0.030	1	0.863
maternal IgE			0.096	1	0.756	0.844	0.037	361.460	1	<0.001	0.051	0.015	11.63	1	0.001
maternal IgG			0.381	1	0.537			0.064	1	0.800	0.181	0.026	39.575	1	<0.001

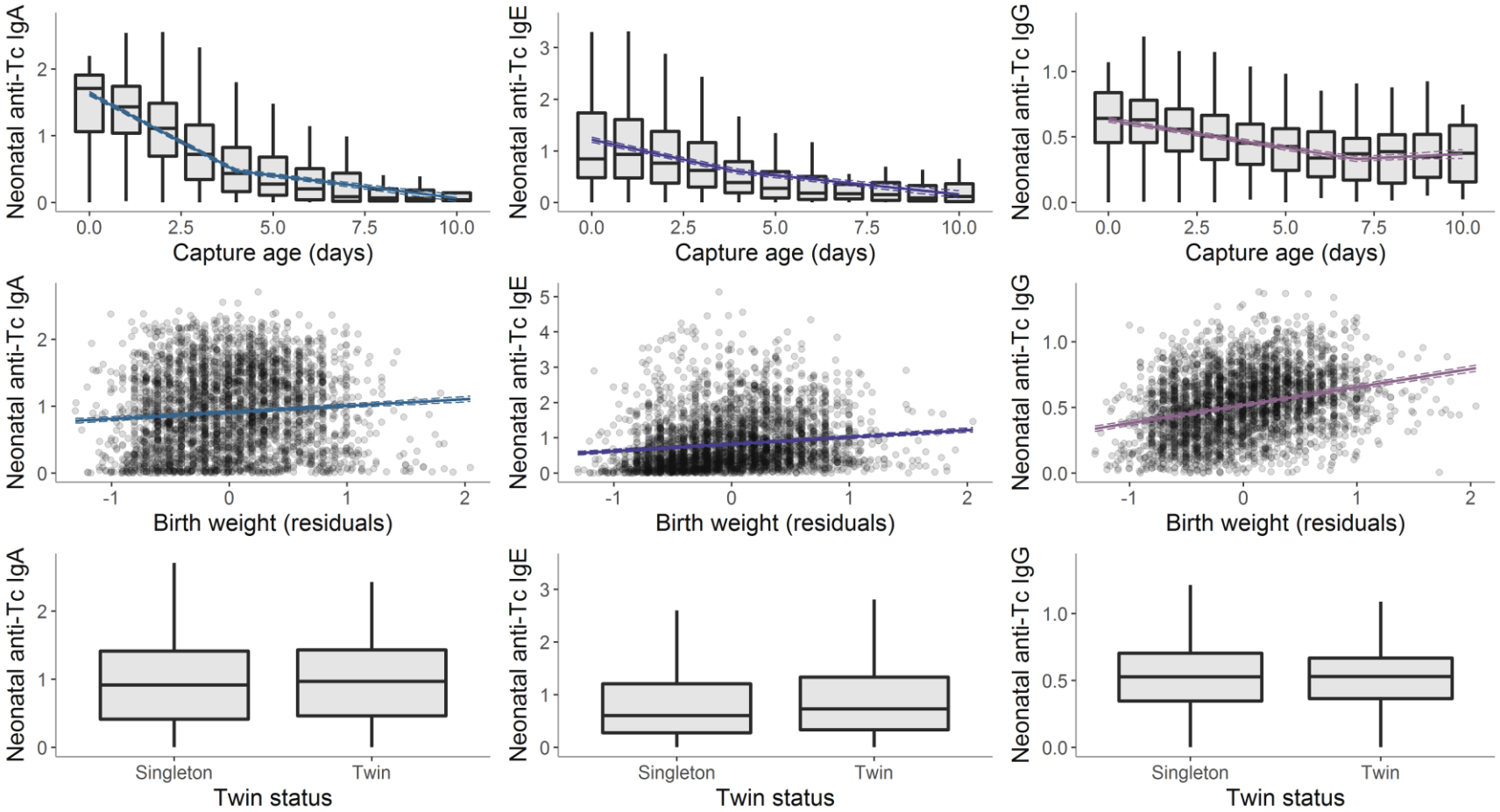

Figure 5.4. Associations between offspring characteristics capture age, birth weight and twin status and neonatal anti-*T. circumcincta* IgA, IgE and IgG levels. Plots show raw data with LMM predictions and standard errors estimated for female singleton lambs with average values for all continuous fixed effects in the minimal model. Boxes show the median and the interquartile range (IQR) with whiskers extending from the hinges to values no further than 1.5*IQR from the hinge (outliers not shown).

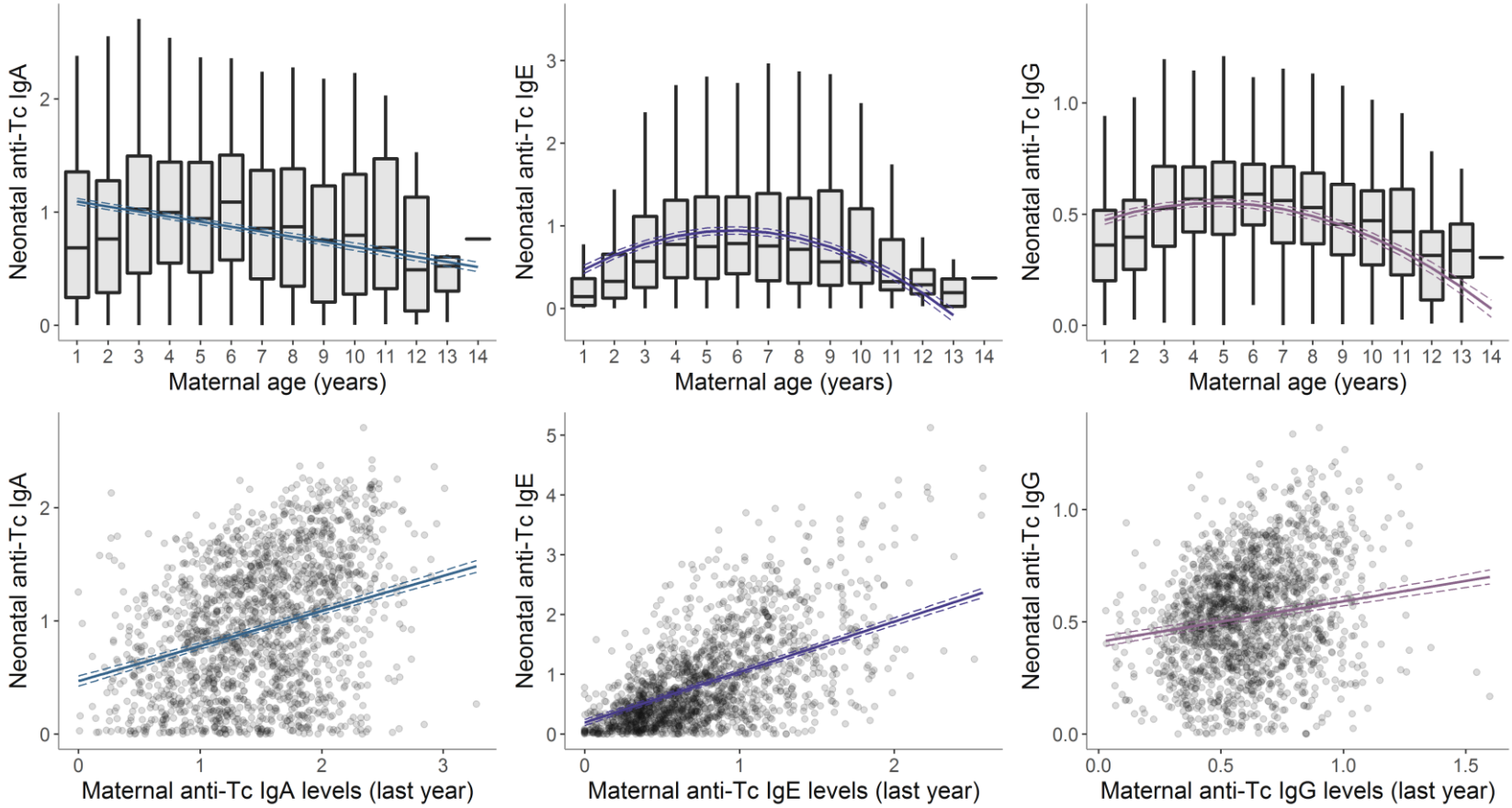

Figure 5.5. Associations between maternal characteristics age and maternal anti-*T. circumcincta* antibody levels and neonatal anti-*T. circumcincta* IgA, IgE and IgG levels. Plots show raw data with LMM predictions and standard errors estimated for female singleton lambs with average values for all continuous fixed effects in the minimal model. Boxes show the median and the interquartile range (IQR) with whiskers extending from the hinges to values no further than 1.5*IQR from the hinge (outliers not shown).

5.4.2 Animal model

There was no evidence for a significant direct additive genetic effect on neonatal anti-*T. circumcincta* antibody levels in any isotype (Table 5.2, Figure 5.6). There was a considerable maternal effects explaining neonatal anti-*T. circumcincta* antibody levels, with maternal identity explaining 48%, 59% and 34% of proportion of the phenotypic variance in anti-Tc IgA, IgE and IgG levels respectively (Table 5.2, Figure 5.6). Evidence for genetic variance underlying neonatal antibody levels was observed as a significant maternal genetic effect, and the proportion of phenotypic variance explained by maternal genetic variance was 0.28±0.05 SE in IgA, 0.41±0.05 in IgE and 0.11±0.04 in IgG. This maternal genetic component explained 57%, 70% and 32% respectively of the maternal effect for IgA, IgE and IgG respectively (Table 5.2, Figure 5.6).

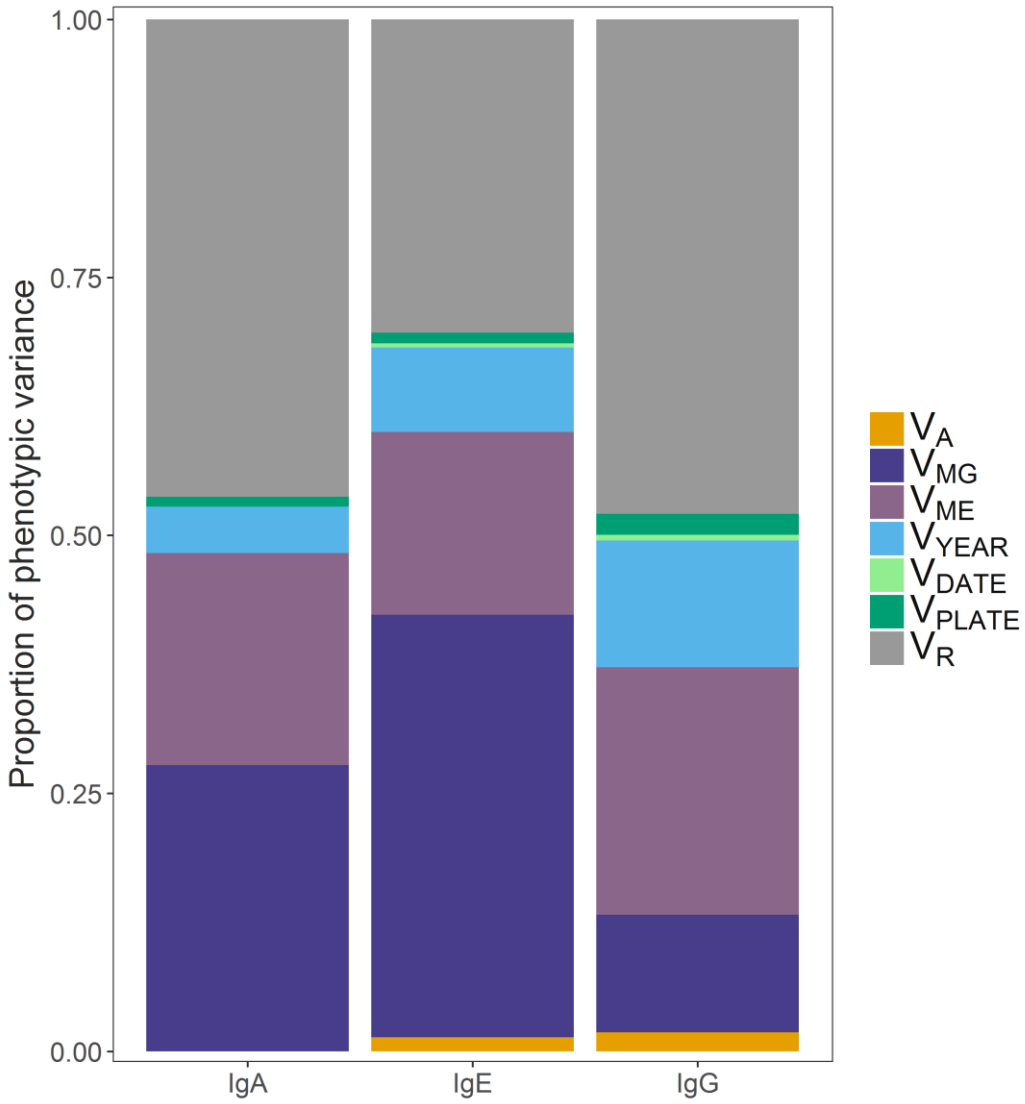

Figure 5.6. The proportion of phenotypic variance explained by different random effects in univariate animal models for neonatal anti-*T. circumcincta* IgA, IgE and IgG levels in wild Soay sheep. Random effects include the following variance components: additive genetic (V_A), maternal genetic (V_{MG}), maternal environment (V_{ME}), birth year (V_{YEAR}), run date of the ELISA assay (V_{DATE}), plate number of the ELISA assay (V_{PLATE}) and the residual error (V_R).

Table 5.2. Variance component estimates and their associated ratios for models of neonatal anti-*T. circumcincta* IgA, IgE and IgG immunoglobulin levels measured in St. Kilda Soay sheep. Variances reported are the additive genetic variance (V_A), maternal genetic variance (V_{MG}), maternal environment variance (V_{ME}), year variance (V_{Year}), ELISA plate variance (V_{Plate}), ELISA run date variance (V_{Date}) and residual variance (V_R). Included are the raw variance component estimates ('Est') and the proportion of the total phenotypic variance explained by the term ('Prop') and their associated standard errors in brackets. The significance of random effects was determined by dropping each random effect from a model containing all random effects and performing a likelihood ratio test on the two log likelihoods ('LRT' and 'p-value'). [B] indicates where variance components went to boundary in the model.

	IgA					**IgE**					**IgG**				
	Est	**Prop**	**LRT**	**d.f.**	**p-value**	**Est**	**Prop**	**LRT**	**d.f.**	**p-value**	**Est**	**Prop**	**LRT**	**d.f.**	**p-value**
	n=3216					n=3221					n=3215				
V_A	<0.001 (<0.001)[B]	<0.001 (<0.001)[B]	N/A	N/A	N/A	0.007 (0.006)	0.014 (0.011)	2.693	1	0.101	0.001 (0.001)	0.018 (0.017)	1.880	1	0.170
V_{MG}	0.070 (0.013)	0.278 (0.048)	**57.432**	**1**	**<0.001**	0.214 (0.032)	0.409 (0.052)	**89.594**	**1**	**<0.001**	0.007 (0.002)	0.114 (0.038)	**11.604**	**1**	**0.001**
V_{ME}	0.052 (0.010)	0.205 (0.042)	**28.268**	**1**	**<0.001**	0.092 (0.021)	0.177 (0.043)	**24.318**	**1**	**<0.001**	0.014 (0.002)	0.240 (0.037)	**46.946**	**1**	**<0.001**
V_{Year}	0.011 (0.004)	0.045 (0.015)	**78.201**	**1**	**<0.001**	0.043 (0.014)	0.082 (0.025)	**111.305**	**1**	**<0.001**	0.007 (0.002)	0.122 (0.034)	**230.967**	**1**	**<0.001**
V_{Plate}	0.002 (0.001)	0.009 (0.005)	**9.391**	**1**	**0.002**	0.005 (0.003)	0.010 (0.006)	**9.894**	**1**	**0.002**	0.001 (0.001)	0.020 (0.012)	**13.920**	**1**	**<0.001**
V_{Date}	<0.001 (<0.001)[B]	<0.001 (<0.001)[B]	N/A	N/A	N/A	0.002 (0.003)	0.004 (0.006)	0.547	1	0.460	<0.001 (0.001)	0.006 (0.012)	0.286	1	0.593
V_R	0.117 (0.003)	0.463 (0.020)	-	-	-	0.158 (0.006)	0.304 (0.018)	-	-	-	0.028 (0.001)	0.479 (0.028)	-	-	-

5.4.3 Consequences of variation in neonatal antibody levels for offspring

Correlations between neonatal anti-*T. circumcincta* antibody levels and endogenous anti-*T. circumcincta* antibody levels at 4 months old of the same isotype were weak. However, there was a significant negative correlation between neonatal and August anti-*T. circumcincta* IgG levels (Figure 5.7). There was a positive linear association between all three neonatal anti-Tc antibody isotypes and August weight, but only IgA and IgG levels predicted August weight independent of neonatal total IgG levels and August antibody levels (Table 5.3, Figure 5.8). In a model including both neonatal anti-Tc IgA and IgG levels, in addition to neonatal total IgG and August antibody measures, all neonatal antibody measures were significant. This suggests that individuals that had higher maternally-derived total IgG, anti-*T. circumcincta* IgG or anti-*T. circumcincta* IgA levels were heavier as lambs in August, independent of birth weight and lamb August antibody levels (Anti-Tc IgA: $\chi^2_{(1)} = 5.165$, p = 0.023; Anti-Tc IgG: $\chi^2_{(1)} = 9.411$, p = 0.002; Total IgG: $\chi^2_{(1)} = 5.946$, p = 0.015). Although neonatal anti-Tc IgG did not predict lamb August FEC when fitted alone, it became significant when total neonatal IgG was included (Table 5.3, Figure 5.8). This result was independent of lamb August anti-*T. circumcincta* IgG levels (Table 5.3). This implies that neonates that had high maternally derived anti-Tc IgG relative to total IgG levels tended to have lower FEC at 4 months old. In contrast, neonates that had high maternally derived total IgG relative to anti-Tc IgG had higher FEC at 4 months old (Table 5.3, Figure 5.8).

There were positive associations between neonatal anti-Tc IgG levels and survival to four months, independent of total IgG levels which did not predict survival over this period (Table 5.4, Figure 5.8). Neonatal anti-Tc IgG levels significantly predicted first winter survival, with lambs with higher anti-Tc IgG levels more likely to survive to the following spring independent of neonatal total IgG levels (Table 5.4, Figure 5.8). This association is likely to be acting through a reduction in August strongyle FEC, since inclusion of FEC in the model caused anti-Tc IgG to no longer be a significant predictor of winter survival ($\chi^2_{(1)} = 1.915$, p = 0.166). Neonatal total IgG levels alone were not associated with first winter survival (Table 5.4).

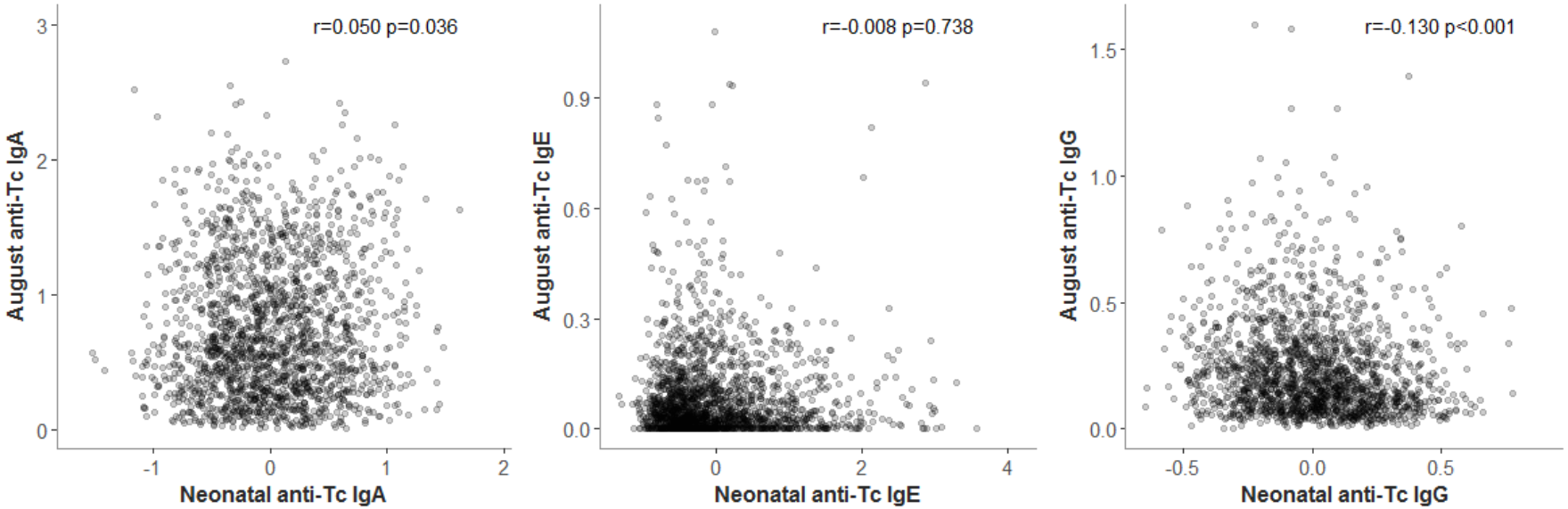

Figure 5.7. Scatterplots of raw data showing correlations between neonatal anti-*T. circumcincta* IgA, IgE, IgG levels (as residuals corrected for capture age see results) and anti-*T. circumcincta* IgA, IgE, IgG levels in August when lambs were around 4 months old. Correlation coefficients (r) and p-values quoted are from Pearson correlation tests.

Table 5.3. LMM and GLMM results of the final minimal model for August weight and strongyle FEC respectively for lambs including the estimated effects (estimate), standard error (SE) and the significance of fixed effects based on a likelihood ratio test (LRT, d.f., p-value). For the added fixed effects, each neonatal antibody measure was added separately to the minimal model and significance tested alone, then tested with a model including neonatal total IgG, followed by a model with August antibody levels. Where the quadratic terms were significant, estimated effects are stated for both the linear and quadratic terms combined.

	August weight n=1783					**August FEC** n=1531				
	estimate	SE	LRT	d.f.	p-value	estimate	SE	LRT	d.f.	p-value
fixed effects										
intercept	2.857	0.826				4.048	0.351			
sex (male)	1.129	0.073	219.830	1	<0.001	0.432	0.042	102.720	1	<0.001
twin	-1.446	0.127	125.560	1	<0.001	0.074	0.061	1.480	1	0.224
birth weight	2.458	0.100	517.520	1	<0.001					
age (days)	0.068	0.007	94.491	1	<0.001					
weight						-0.348	0.054	-	-	-
weight^2						0.010	0.002	23.600	1	<0.001
maternal age	0.860	0.073	-	-	-	0.061	0.038	-	-	-
maternal age^2	-0.076	0.006	156.170	1	<0.001	-0.004	0.003	1.460	1	0.227
added fixed effects										
IgA										
neonatal anti-Tc IgA levels	**0.510**	**0.086**	**34.811**	**1**	**<0.001**	-0.051	0.043	1.420	1	0.233
neonatal anti-Tc IgA levels	**0.269**	**0.094**	**8.264**	**1**	**0.004**	-0.071	0.046	2.400	1	0.121
neonatal total IgG levels	**0.974**	**0.160**	**36.767**	**1**	**<0.001**	0.108	0.085	1.580	1	0.209
neonatal anti-Tc IgA levels	**0.261**	**0.094**	**7.750**	**1**	**0.005**	-0.046	0.046	1.020	1	0.313
neonatal total IgG levels	**0.991**	**0.161**	**37.275**	**1**	**<0.001**	0.059	0.086	0.480	1	0.488
August anti-Tc IgA levels	0.068	0.078	0.749	1	0.387	**-0.194**	**0.042**	**21.620**	**1**	**<0.001**

IgE										
neonatal anti-Tc IgE levels	**0.310**	**0.064**	**23.208**	**1**	**<0.001**	0.020	0.030	0.440	1	0.507
neonatal anti-Tc IgE levels	0.096	0.071	1.858	1	0.173	0.014	0.033	0.160	1	0.689
neonatal total IgG levels	**1.064**	**0.163**	**41.999**	**1**	**<0.001**	0.045	0.088	0.260	1	0.610
neonatal anti-Tc IgE levels	0.088	0.071	1.570	1	0.210	0.018	0.033	0.300	1	0.584
neonatal total IgG levels	**1.103**	**0.164**	**44.808**	**1**	**<0.001**	0.043	0.088	0.240	1	0.624
August anti-Tc IgE levels	**0.897**	**0.265**	**11.498**	**1**	**0.001**	**-0.427**	**0.149**	**8.240**	**1**	**0.004**
IgG										
neonatal anti-Tc IgG levels	**1.530**	**0.185**	**67.637**	**1**	**<0.001**	-0.120	0.096	1.560	1	0.212
neonatal anti-Tc IgG levels	**0.940**	**0.274**	**11.761**	**1**	**0.001**	**-0.353**	**0.137**	**6.540**	**1**	**0.011**
neonatal total IgG levels	**0.620**	**0.216**	**8.248**	**1**	**0.004**	**0.272**	**0.115**	**5.620**	**1**	**0.018**
neonatal anti-Tc IgG levels	**0.935**	**0.274**	**11.603**	**1**	**0.001**	**-0.397**	**0.138**	**8.140**	**1**	**0.004**
neonatal total IgG levels	**0.635**	**0.217**	**8.604**	**1**	**0.003**	**0.277**	**0.115**	**5.780**	**1**	**0.016**
August anti-Tc IgG levels	0.280	0.209	1.785	1	0.182	**-0.473**	**0.112**	**17.800**	**1**	**<0.001**

Table 5.4. GLMM results of the final minimal model for neonate and first winter survival including the estimated effects (estimate), standard error (SE) and the significance of fixed effects based on a likelihood ratio test (LRT, d.f., p-value). For the added fixed effects, each neonatal antibody measure was added separately to the minimal model and significance tested alone, then tested with a model including neonatal total IgG, followed by a model with August antibody levels. Where interaction terms are significant, estimated effects are stated for the individual terms with the interaction term in the model.

	Neonate survival n=3228					**Winter survival** n=1647				
	estimate	SE	LRT	d.f.	p-value	estimate	SE	LRT	d.f.	p-value
fixed effects										
intercept	4.2746	0.3153				0.4307	0.3788			
twin	0.6563	0.2854	5.461	1	0.019					
sex (male)	-0.3169	0.192	2.753	1	0.097	-1.0145	0.1448	-	-	-
birth weight	0.9584	0.1419	56.384	1	<0.001					
maternal age	0.4214	0.3767	-	-	-					
maternal age^2	-0.7348	0.3525	4.186	1	0.041					
August weight						1.0686	0.1241	-	-	-
sex (male)* weight						-0.3597	0.1527	5.466	1	0.019
added fixed effects										
IgA										
neonatal anti-Tc IgA levels	**0.383**	**0.114**	**12.210**	**1**	**<0.001**	0.084	0.071	1.359	1	0.244
neonatal anti-Tc IgA levels	0.192	0.125	2.444	1	0.118	0.066	0.075	0.736	1	0.391
neonatal total IgG levels	**0.318**	**0.106**	**8.621**	**1**	**0.003**	0.044	0.077	0.312	1	0.576
neonatal anti-Tc IgA levels						0.084	0.076	1.169	1	0.280
neonatal total IgG levels						0.037	0.079	0.217	1	0.641
August anti-Tc IgA levels						-0.062	0.069	0.775	1	0.379

IgE										
neonatal anti-Tc IgE levels	0.202	0.123	2.852	1	0.091	0.067	0.069	0.908	1	0.341
neonatal anti-Tc IgE levels	-0.091	0.133	0.462	1	0.497	0.046	0.077	0.349	1	0.555
neonatal total IgG levels	**0.443**	**0.107**	**16.498**	**1**	**<0.001**	0.045	0.080	0.307	1	0.580
neonatal anti-Tc IgE levels						0.043	0.077	0.306	1	0.581
neonatal total IgG levels						0.058	0.081	0.499	1	0.480
August anti-Tc IgE levels						0.026	0.069	0.131	1	0.718
IgG										
neonatal anti-Tc IgG levels	**0.562**	**0.112**	**26.087**	**1**	**<0.001**	**0.167**	**0.073**	**5.176**	**1**	**0.023**
neonatal anti-Tc IgG levels	**0.479**	**0.187**	**6.802**	**1**	**0.009**	**0.247**	**0.104**	**5.575**	**1**	**0.018**
neonatal total IgG levels	0.086	0.152	0.320	1	0.572	-0.114	0.104	1.176	1	0.278
neonatal anti-Tc IgG levels						**0.243**	**0.104**	**5.324**	**1**	**0.021**
neonatal total IgG levels						-0.100	0.105	0.883	1	0.348
August anti-Tc IgG levels						0.007	0.072	0.011	1	0.918

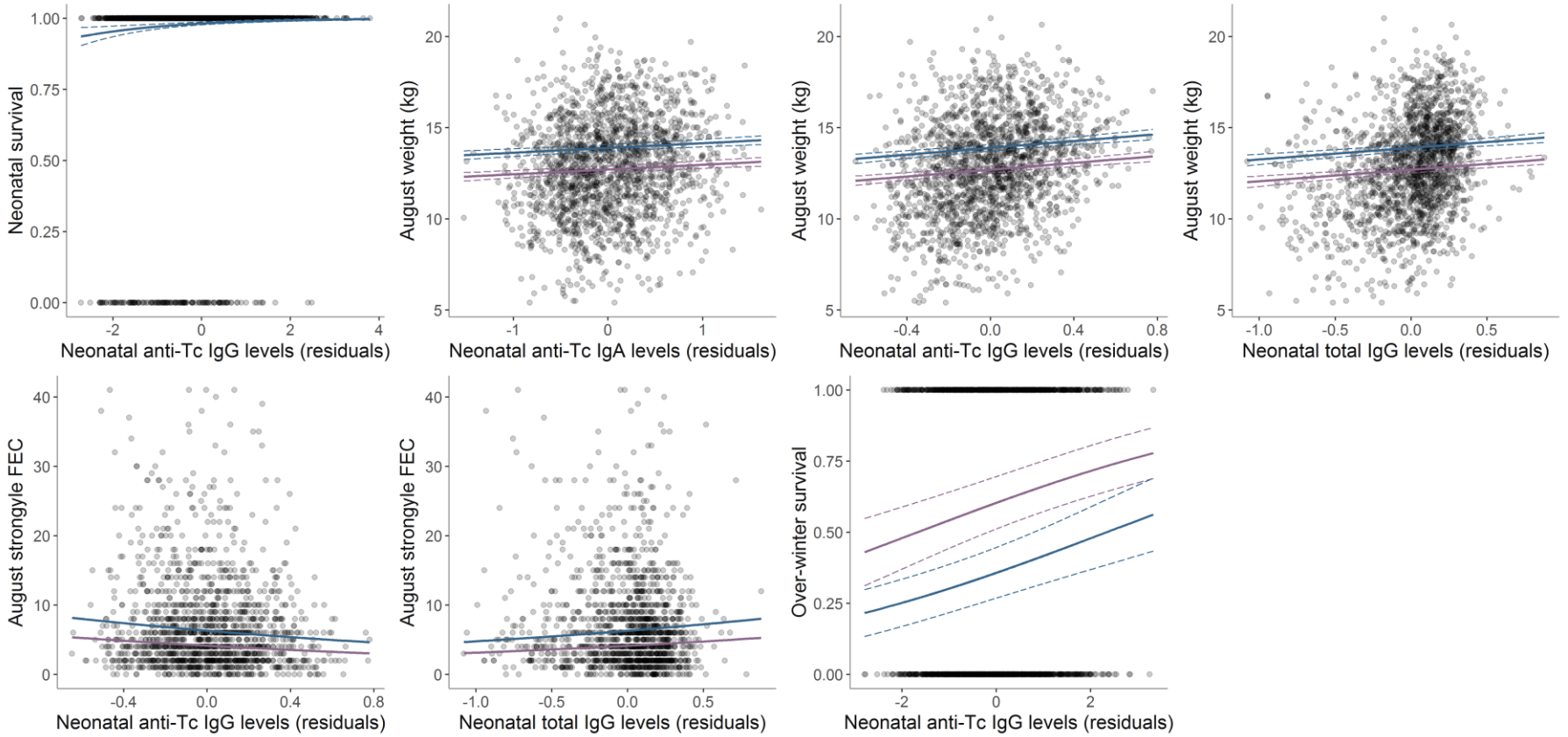

Figure 5.8. Associations between neonatal anti-Tc antibody levels and neonatal survival, August weight, August strongyle FEC and over-winter survival. Plots show raw data with LMM or GLMM predictions and standard errors estimated for singleton lambs for males (blue) and females (purple) with average values for all continuous fixed effects in a model containing neonatal total IgG and August antibody levels.

5.4.4 Consequences of variation in neonatal antibody levels for mothers

There was evidence for a positive association between maternally transferred anti-*T. circumcincta* IgG levels and the mother's subsequent weight. However, after accounting for the mother's weight in the previous year this association was no longer significant, which suggests that the previous positive association may simply be due to heavier mothers tending to have offspring with higher neonatal antibody levels (Table 5.5-5.6). We found no association between neonatal anti-*T. circumcincta* IgA, IgE, IgG or total IgG levels and the mother's subsequent FEC level either including or excluding her FEC level in the previous year (Table 5.5-5.6).

There were difficulties with model convergence when trying to fit the binomial GLMMs of maternal fitness due to the high proportion of mothers surviving and breeding in the subsequent year (Table 5.7). The base models of winter survival did not converge, and the models of breeding success with neonatal anti- *T. circumcincta* IgG, total IgG and August anti-*T. circumcincta* IgG also did not converge. Due to the problems with model convergence these results should be treated with caution.

Table 5.5. LMM results of the final minimal model for mother's weight and strongyle FEC in the August following lambing without including mother's weight and strongyle FEC in the previous year. The table includes the estimated effects (estimate), standard error (SE) and the significance of fixed effects based on a likelihood ratio test (LRT, d.f., p-value). For the added fixed effects, each neonatal antibody measure was added separately to the minimal model and significance tested alone, then tested with a model including neonatal total IgG, followed by a model with mother's August antibody levels. Where the quadratic terms were significant, estimated effects are stated for both the linear and quadratic terms combined.

	Maternal weight n=1741					**Maternal FEC** n=1585				
	estimate	SE	LRT	d.f.	p-value	estimate	SE	LRT	d.f.	p-value
fixed effects										
intercept	18.058	0.229				6.638	1.400			
age	1.502	0.068	-	-	-	-0.291	0.063	-	-	-
age^2	-0.107	0.005	330.100	1	<0.001	0.024	0.005	20.560	1	<0.001
lamb birth weight	0.816	0.092	77.406	1	<0.001					
lamb twin status (twin)	0.525	0.120	19.102	1	<0.001	-0.208	0.099	4.460	1	0.035
lamb sex (male)						0.138	0.067	4.180	1	0.041
weight this year						-0.461	0.129	-	-	-
weight this year^2						0.008	0.003	8.280	1	0.004
added fixed effects										
IgA										
neonatal anti-Tc IgA levels	0.157	0.085	3.401	1	0.065	0.017	0.075	0.060	1	0.807
neonatal anti-Tc IgA levels	0.127	0.098	1.686	1	0.194	0.074	0.081	0.840	1	0.359
neonatal total IgG levels	0.099	0.149	0.449	1	0.503	-0.248	0.145	2.940	1	0.086
neonatal anti-Tc IgA levels	0.068	0.100	0.459	1	0.498	0.164	0.090	3.280	1	0.070
neonatal total IgG levels	0.138	0.149	0.852	1	0.356	**-0.316**	**0.148**	**4.540**	**1**	**0.033**
August anti-Tc IgA levels	**0.305**	**0.115**	**7.025**	**1**	**0.008**	**-0.189**	**0.082**	**5.320**	**1**	**0.021**

IgE										
neonatal anti-Tc IgE levels	0.063	0.071	0.773	1	0.379	0.046	0.056	0.660	1	0.417
neonatal anti-Tc IgE levels	0.004	0.084	0.002	1	0.961	0.105	0.063	2.740	1	0.098
neonatal total IgG levels	0.205	0.152	1.827	1	0.177	**-0.297**	**0.149**	**3.960**	**1**	**0.047**
neonatal anti-Tc IgE levels	-0.039	0.091	0.189	1	0.664	0.083	0.079	1.080	1	0.299
neonatal total IgG levels	0.230	0.154	2.233	1	0.135	-0.280	0.154	3.280	1	0.070
August anti-Tc IgE levels	0.178	0.152	1.374	1	0.241	0.053	0.117	0.200	1	0.655
IgG										
neonatal anti-Tc IgG levels	**0.458**	**0.181**	**6.393**	**1**	**0.011**	-0.244	0.161	2.300	1	0.129
neonatal anti-Tc IgG levels	**0.632**	**0.292**	**4.627**	**1**	**0.031**	-0.151	0.245	0.380	1	0.538
neonatal total IgG levels	-0.155	0.210	0.538	1	0.464	-0.098	0.201	0.240	1	0.624
neonatal anti-Tc IgG levels	**0.624**	**0.308**	**4.061**	**1**	**0.044**	0.134	0.291	0.220	1	0.639
neonatal total IgG levels	-0.149	0.216	0.463	1	0.497	-0.262	0.220	1.420	1	0.233
August anti-Tc IgG levels	-0.020	0.252	0.007	1	0.934	-0.420	0.230	3.360	1	0.067

Table 5.6. LMM results of the final minimal model for mother's weight and strongyle FEC in the August following lambing including mother's weight and strongyle FEC in the previous year. The table includes the estimated effects (estimate), standard error (SE) and the significance of fixed effects based on a likelihood ratio test (LRT, d.f., p-value). For the added fixed effects, each neonatal antibody measure was added separately to the minimal model and significance tested alone, then tested with a model including neonatal total IgG, followed by a model with mother's August antibody levels. Where the quadratic terms were significant, estimated effects are stated for both the linear and quadratic terms combined.

	Maternal weight n=1146					**Maternal FEC** n=959				
	estimate	SE	LRT	d.f.	p-value	estimate	SE	LRT	d.f.	p-value
fixed effects										
intercept	8.658	0.333				8.008	1.777			
age	-0.143	0.022	36.944	1	<0.001	-0.206	0.081	-	-	-
age^2						0.018	0.006	7.480	1	0.006
weight last year	0.660	0.017	793.180	1	<0.001					
weight this year						-0.630	0.164	-	-	-
weight this year^2						0.013	0.004	12.060	1	0.001
FEC last year						0.072	0.026	8.080	1	0.004
lamb twin status (twin)						-0.250	0.123	4.200	1	0.040
added fixed effects										
IgA										
neonatal anti-Tc IgA levels	0.012	0.089	0.017	1	0.898	0.036	0.093	0.160	1	0.689
neonatal anti-Tc IgA levels	0.039	0.097	0.156	1	0.693	0.074	0.100	0.540	1	0.462
neonatal total IgG levels	-0.119	0.164	0.513	1	0.474	-0.177	0.188	0.880	1	0.348
neonatal anti-Tc IgA levels	-0.013	0.106	0.016	1	0.900	0.107	0.114	0.900	1	0.343
neonatal total IgG levels	-0.078	0.168	0.221	1	0.638	-0.208	0.195	1.140	1	0.286
August anti-Tc IgA levels	0.119	0.100	1.435	1	0.231	-0.069	0.104	0.440	1	0.507

IgE										
neonatal anti-Tc IgE levels	-0.128	0.065	3.670	1	0.055	0.014	0.072	0.040	1	0.842
neonatal anti-Tc IgE levels	**-0.147**	**0.074**	**3.887**	**1**	**0.049**	0.045	0.081	0.320	1	0.572
neonatal total IgG levels	0.091	0.168	0.279	1	0.598	-0.155	0.194	0.640	1	0.424
neonatal anti-Tc IgE levels	-0.130	0.092	2.011	1	0.156	0.064	0.100	0.400	1	0.527
neonatal total IgG levels	0.077	0.173	0.188	1	0.665	-0.171	0.199	0.740	1	0.390
August anti-Tc IgE levels	-0.047	0.143	0.106	1	0.745	-0.051	0.153	0.100	1	0.752
IgG										
neonatal anti-Tc IgG levels	0.054	0.189	0.070	1	0.791	-0.139	0.204	0.460	1	0.498
neonatal anti-Tc IgG levels	0.276	0.284	0.926	1	0.336	-0.067	0.307	0.040	1	0.842
neonatal total IgG levels	-0.245	0.225	1.168	1	0.280	-0.075	0.261	0.080	1	0.777
neonatal anti-Tc IgG levels	0.355	0.327	1.157	1	0.282	0.209	0.370	0.320	1	0.572
neonatal total IgG levels	-0.295	0.243	1.456	1	0.228	-0.247	0.288	0.740	1	0.390
August anti-Tc IgG levels	-0.138	0.257	0.286	1	0.593	-0.377	0.284	1.780	1	0.182

Table 5.7. GLMM results of the final minimal model for mother's breeding success following lambing including the estimated effects (estimate), standard error (SE) and the significance of fixed effects based on a likelihood ratio test (LRT, d.f., p-value). For the added fixed effects, each neonatal antibody measure was added separately to the minimal model and significance tested alone, then tested with a model including neonatal total IgG, followed by a model with mother's August antibody levels. Where the quadratic terms were significant, estimated effects are stated for both the linear and quadratic terms combined. DNC indicates models that did not converge.

	Maternal breeding success n=1481				
	estimate	SE	LRT	d.f.	p-value
fixed effects					
intercept	9.875	0.907			
age	-0.930	0.288	11.407	1	0.001
weight	0.116	0.371	0.117	1	0.733
weight^2					
lamb birth weight					
added fixed effects					
IgA					
neonatal anti-Tc IgA levels	**-0.737**	**0.321**	**5.242**	**1**	**0.022**
neonatal anti-Tc IgA levels	-0.551	0.350	2.484	1	0.115
neonatal total IgG levels	-0.443	0.315	2.029	1	0.154
neonatal anti-Tc IgA levels	-0.609	0.367	2.810	1	0.094
neonatal total IgG levels	-0.424	0.314	1.906	1	0.167
August anti-Tc IgA levels	0.252	0.388	0.401	1	0.526
IgE					
neonatal anti-Tc IgE levels	-0.148	0.411	0.194	1	0.660
neonatal anti-Tc IgE levels	0.263	0.425	0.386	1	0.535
neonatal total IgG levels	**-0.693**	**0.323**	**5.029**	**1**	**0.025**
neonatal anti-Tc IgE levels	0.083	0.519	0.014	1	0.907
neonatal total IgG levels	-0.653	0.331	3.064	1	0.080
August anti-Tc IgE levels	0.273	0.485	0.310	1	0.578
IgG					
neonatal anti-Tc IgG levels	-0.206	0.288	0.506	1	0.477
neonatal anti-Tc IgG levels	0.813	0.506	2.707	1	0.100
neonatal total IgG levels	**-1.164**	**0.452**	**7.021**	**1**	**0.008**
neonatal anti-Tc IgG levels	DNC	DNC	DNC	DNC	DNC
neonatal total IgG levels	DNC	DNC	DNC	DNC	DNC
August anti-Tc IgG levels	DNC	DNC	DNC	DNC	DNC

5.5 Discussion

In this study we investigated the causes and consequences of variation in maternally transferred antibody levels in a wild Soay sheep population. We found that offspring characteristics, including birth weight, twin status and capture age, were significantly associated with neonatal antibody levels. Maternal age and antibody levels in the previous year were also significantly associated with neonatal antibody levels. There was evidence for maternal genetic variance, but not offspring additive genetic variance, in neonatal anti-*T. circumcincta* antibody levels. We also found that previous associations with anti-*T. circumcincta* IgA and IgE and weight and neonate survival in Chapter 4 were likely driven by associations with anti-*T. circumcincta* IgG or total IgG levels. We have shown that neonatal parasite-specific IgG levels were associated with weight and parasite faecal egg count, and over winter survival via this reduction in parasite burden, independent of total IgG levels. These results suggest that the quality of the colostrum, in addition to antibody levels to an ecologically relevant parasite transferred by mothers, are associated with offspring phenotype and fitness.

In concordance with Chapter 4, we found that capture age, twin status, birth weight and maternal age were significantly associated with neonatal anti-*T. circumcincta* antibody levels. However, while levels of neonatal anti-*T. circumcincta* IgA and IgE declined with increasing capture age, there was a decline in anti-*T. circumcincta* IgG levels up to 7 days old after which there was no association with capture age, likely due to the longer half-life of maternally-transferred IgG antibodies (Husband *et al.* 1972). The association of higher neonatal antibody levels with heavier born lambs, twins and prime-aged mothers may be due to their association with mothers in better condition (Clutton-Brock & Pemberton 2004). These results are consistent with literature in domestic ruminants (discussed in Chapter 4), in addition to eco-immunological literature that suggests that maternal antibody transfer is closely related to maternal condition (Morales *et al.* 2006; Hargitai *et al.* 2006) and nutrition (Pihlaja *et al.* 2006; Karell *et al.* 2008). We also found that mother's plasma antibody levels were positively associated with her offspring's neonatal antibody levels in the following year. In ruminants, all of the IgG and half of the IgA in colostrum are derived from the bloodstream (Tizard 2012). Due to the parasite-specificity of these measures, it is likely that these antibodies were produced in mucosal sites but, rather than being secreted, were transported from the blood to the colostrum via the polymeric immunoglobulin receptor

(pIgR) or the neonatal Fc receptor (FcRn) or CD23 on mammary epithelial cells (Pastoret *et al.* 1998; Butler 1999; Mayer *et al.* 2002; Hine *et al.* 2010). The association of neonatal antibody levels with levels in the mother, indicate that antibodies received by the neonate are indicative of the mother's disease history, in addition to potentially being subject to the costs associated with eliciting and maintaining an energetically demanding immune response (Demas & Nelson 2012).

To the best of our knowledge, other than the pilot study in Chapter 4, no studies in the wild have previously partitioned variance in maternally-transferred antibody levels into additive genetic, maternal environment and maternal genetic components. We have shown that neonatal antibody levels are not directly heritable in terms of an offspring additive genetic component. Instead, there is a large maternal effect explaining variance in neonatal antibody levels suggesting that mothers are consistent in the levels of maternal antibodies transferred. This is in contrast with the results from Chapter 4 which found that neonatal anti-Tc IgE levels, but not IgA levels, were significantly heritable. However, the dataset used in this chapter is around 5 times the size of the dataset used in the preceding chapter and the large standard errors around the previous heritability estimate indicate this. As a result, in addition to what is known about the biology of maternal antibody transfer in ruminants (Butler 1999), it is highly likely that there is no, or low, direct additive genetic variance. Previously, the consistency of mothers in maternal antibody transfer has been observed in birds (Coakley *et al.* 2014), while in cows the total IgG content in colostrum has also been found to be repeatable (Dardillat *et al.* 1978; Norman *et al.* 1981). A substantial proportion of these maternal effects, between 32-70%, were genetically based. Variance in other traits in Soay sheep (birth weight and birth date) have also been found to be explained by maternal genetic, but not additive genetic, effects (Wilson *et al.* 2005). The lack of additive genetic variance implies no genetic antagonism between mother and offspring in this trait. This could potentially be due to strong selection removing all additive genetic variance underlying the variation in neonatal antibody levels at the offspring level or may be the result of parental-offspring conflict being resolved entirely to parental control. As a result of this, selection can only act on the mother, and any genetic change in the maternal trait will affect offspring phenotype and fitness (Räsänen & Kruuk 2007). Strong selection on a trait is likely to reduce underlying genetic variation and hence heritability of quantitative traits (Falconer & Mackay 1996). The presence of fitness benefits for the offspring is as expected with the lack of direct

additive genetic variance, while the lack of clear maternal costs is consistent with the considerable maternal genetic effect.

Previous studies in livestock have found evidence of genetic influences on maternal antibody transfer (Laegreid *et al.* 2002; Clawson *et al.* 2004; Rohrer *et al.* 2014). In a study of serum immunocrit levels in neonatal pigs the maternal genetic effect was four times the size of the direct offspring additive genetic effect (Rohrer *et al.* 2014). Despite this, efforts to identify candidate genes for neonatal antibody levels have focussed primarily on genetic polymorphisms in neonatal calves and piglets rather than in the mothers (Clawson *et al.* 2004; Rohrer *et al.* 2014). In calves, failure of immune transfer has been associated with candidate genes beta 2 microglobulin (B2M) (Laegreid *et al.* 2002) and the gene encoding the α chain of the FcRn receptor (FCGRT) (Clawson *et al.* 2004), while in piglets SNPs have been identified close to candidate genes associated with appetite (Rohrer *et al.* 2014). Considering the large economic costs of failure of passive immune transfer in ruminants, in addition to the contribution of overuse of antimicrobials (Raboisson, Trillat & Cahuzac 2016), our study suggests the genetic basis of neonatal antibody levels should be searched for in mothers and selective breeding on offspring genotype or breeding value may have very little effect.

A particularly important part of this study was the measurement of both total and parasite-specific IgG levels. Previously, in Chapter 4, we found positive associations between anti-*T. circumcincta* IgA and IgE levels with weight, and IgE levels with neonate survival. Using these new IgG isotype measures, we were able to determine that these previous associations were due to associations with anti-*T. circumcincta* IgG or total IgG levels. We found that neonatal anti-*T. circumcincta* IgA and total IgG levels were positively associated with weight at 4 months old, the latter indicative of general nutritional quality having a positive impact on offspring growth. Neonatal serum total IgG levels are largely associated with improved growth and survival in ruminants (Robison *et al.* 1988; Wittum & Perino 1995). We also found evidence for benefits of anti-*T. circumcincta* IgG levels, independent of total IgG, as they were positively associated with neonate survival, in addition to weight and negatively associated with strongyle FEC at 4 months old. Interestingly, the negative association between anti-*T. circumcincta* IgG levels and FEC was only significant when total IgG was in the model, implying that the levels of anti-*T. circumcincta* IgG relative to total IgG is important. We also found that neonates with higher anti-*T. circumcincta* IgG levels

were more likely to survive their first winter, likely via reductions in strongyle FEC levels. These results show that maternally-transferred parasite-specific antibody levels can have both short-term fitness benefits for the offspring, in addition to longer-term fitness effects through effects on offspring phenotype. The negative association between neonatal anti-parasite IgG levels and strongyle faecal egg counts suggests that these maternally transferred antibodies may result in protective immune transfer which results in reducing lamb worm burden. Previously we found that a lamb's endogenous anti-*T. circumcincta* IgA levels were associated with reduced FEC but found no associations with any endogenously produced antibody levels and over-winter survival (Chapter 3). The presence of an independent association between maternally transferred antibodies and FEC, and a fitness association with survival not found with endogenous antibody levels shows that maternal antibodies can be associated with long-term effects on offspring phenotype and fitness over and above the effects of the offspring's endogenous antibody production.

The associations between neonatal anti-*T. circumcincta* IgG, rather than IgA or IgE, with offspring phenotype and fitness, may be due to the different half-lives of these antibodies. The half-life of passively transferred IgG in neonatal ruminants is estimated to be 16-32 days, compared with only 2.5 days for IgA (Husband *et al.* 1972). However, it is not known whether these half-lives are true biological half-lives or due to selective removal of IgA from the circulation by pIg receptors and secretion at mucosal surfaces (Pastoret *et al.* 1998). As a result, we may be picking up associations with IgG due to the longer half-life of these antibodies, which may in turn have direct immunoprotective effects and could allow more resources available for growth (Demas & Nelson 2012). Alternatively, the longer half-life could mean a longer time to potentially prime the immune response (Husband *et al.* 1972). However, there was a weak negative association between neonatal and August anti-*T. circumcincta* IgG levels, suggesting that maternal antibodies may be blocking rather than priming the endogenous immune response of lambs. Blocking effects have been observed in humans (Siegrist 2003) but evidence for this is rare in wild populations (Staszewski *et al.* 2007). Further work could make use of a multivariate approach to look at the covariance between the lamb's neonatal antibody levels and August antibody levels, and look for evidence of blocking or priming of the immune response. Even after gut closure, immunoglobulins continue to be found in ruminant milk, and have a role in local protection in the gut during lactation (Pastoret *et al.* 1998). If neonatal antibody levels do reflect levels in the colostrum and later levels in the milk, despite immunoglobulin isotype ratio changes,

we could be distinguishing between mothers delivering high and low levels of anti-*T. circumcincta* IgG in milk delivered to the gut during the whole of lactation (Pastoret *et al.* 1998; Butler 1999). Anti-*T. circumcincta* IgG, but not IgA and IgE, is also associated with lower strongyle FEC levels and improved over-winter survival in adults (Chapter 3, Nussey *et al.* 2014; Watson *et al.* 2016). IgG has been documented to have an important role in passive immunity to helminth parasites in laboratory mice (Appleton & McGregor 1987; Harris *et al.* 2006) as well as later development and maintenance of protective immunity to helminths (Blackwell & Else 2001; McCoy *et al.* 2008), and our results suggest that parasite-specific IgG may be particularly important for resistance to strongyle parasites and subsequent fitness in wild Soay sheep.

Since antibody levels in the neonate are associated with the mother's plasma antibody levels, it is likely that maternal antibody transfer may afford the same costs as expected for inducing and eliciting an immune response (Lochmiller & Deerenberg 2000; Schmid-Hempel 2003). However, we found no association with change in weight or strongyle FEC levels between the August preceding and August following lambing. The lack of any association may be due to costs being short-term immediately following lambing, and mothers may have recovered condition by August when grazing is plentiful (Crawley *et al.* 2004). Since mothers can recover their condition by August, it is unlikely that levels of neonatal antibody transferred will have strong fitness effects on the mother. Alternatively, it may be that the dataset already includes only good quality mothers that produced lambs that survived long enough to be caught and bled. We did encounter problems of model convergence with binomial models of over-winter survival and breeding success, likely due to the high proportion of adult females surviving and breeding in the following year. To progress this further, binomial GLMMs should be re-run in a Bayesian package like MCMCglmm or, since variation in these traits is so low, other potential traits that may reveal costs for the mother should be investigated. For instance, it would be interesting to see whether there were any associations between maternal antibody transfer and the mother's own subsequent plasma antibody levels phenotype, in addition to the antibody levels transferred and birth weight of the lamb in the following year.

5.6 Conclusion

This study represents, together with Chapter 4, the first quantification of both the maternal effects, and separation of additive genetic and maternal genetic effects, influencing maternally transferred antibody levels in the wild. By separating these genetic effects, we have shown that maternally transferred antibody levels in wild Soay sheep are associated with genetic variation at the maternal rather than offspring level. We have also shown that while neonatal total IgG and anti-*T. circumcincta* IgA and IgG levels were associated with early growth, anti-*T. circumcincta* IgG was also associated with neonatal survival. In addition, we found that anti-*T. circumcincta* IgG levels had a negative association with strongyle FEC suggesting that there may be a direct transfer of immunity to offspring. The fact that neonatal anti-*T. circumcincta* IgG levels predicted offspring fitness through improved first winter survival via effects on FEC, provides rare evidence for a long-term fitness benefit of maternal antibodies in the wild. While there is a clear link between maternal antibodies and protection, particularly IgG, against helminth parasites in laboratory mice (Appleton & McGregor 1987; Harris *et al.* 2006), studies in domestic animals have typically focussed on total colostrum quality in the form of total IgG (Weaver *et al.* 2000; Gulliksen *et al.* 2008; Bielmann *et al.* 2010) and the transfer of parasite-specific antibody isotypes present has been largely ignored (Pfeffer *et al.* 2005). Our results suggest that parasite-specific IgG levels provided to the neonatal ruminant are important in resistance to gastrointestinal nematode parasites in early life and provide protection over and above the lamb's own endogenous antibodies. However, associations between maternal antibody transfer and potential costs to the mother were unclear and require further analyses. This is a unique long-term dataset on maternally transferred antibodies to an ecologically relevant parasite in a wild mammal, and these results constitute the first study quantifying both genetic and non-genetic sources of variation underlying maternal antibody transfer, and linking maternal antibody transfer with protection to local parasites as well as short and long-term fitness of offspring and mothers.

Chapter 6
General Discussion

6.1 Aims and key findings

The aim of this PhD was to determine the causes and consequences of variation in maternally-transferred and endogenously produced anti-helminth immune responses in the Soay sheep of St Kilda. The main findings presented in this thesis are as follows:

1. Anti-*Teladorsagia circumcincta* IgA, IgE and IgG levels in August are highly repeatable across an individual's lifetime, particularly in adulthood. I found that this high repeatability was in part due to genetic variation among individuals, with all three isotypes being significantly heritable in both lambs and adults. In comparison, only a small amount of variance was explained by maternal, birth year and capture year effects. To identify whether there were particular genes or genomic regions contributing to the genetic variation in these antibody levels, I used a genome wide association study (GWAS) and discovered that anti-*T. circumcincta* IgA levels, but not IgE or IgG, were associated with a quantitative trait locus (QTL). This QTL was associated with a region on chromosome 24, and in this region one single nucleotide polymorphism (SNP) explained 28% of the heritable variation in anti-*T. circumcincta* IgA levels.

2. There were age and isotype specific associations between anti-*T. circumcincta* antibody levels and strongyle faecal egg count (FEC), with low strongyle faecal egg counts predicted by high anti-*T. circumcincta* IgA in lambs but high IgG in adults. Associations between weight and anti-*T. circumcincta* antibody levels were generally positive but were not strong, suggesting immune variation was not just a reflection of host condition. I also found evidence for positive directional selection but on IgG only, with anti-*T. circumcincta* IgG levels positively predicting overwinter survival in adult females, and subsequent fecundity in adult males. I

found no evidence for selection on the SNPs associated with anti-*T. circumcincta* IgA levels.

3. Prime-aged mothers and mothers with high plasma levels of anti-*T. circumcincta* antibodies were more likely to have lambs with high maternally-derived neonatal anti-*T. circumcincta* antibody levels. In addition, high neonatal anti-*T. circumcincta* antibody levels were associated with heavier born lambs, twins, and lambs caught close to birth. There was no additive genetic variance underlying neonatal antibody levels, but maternal, and maternal genetic effects, explained a considerable proportion of the variance in these traits. Positive associations with offspring phenotype and fitness were mainly observed with neonatal anti-*T. circumcincta* IgG levels. Anti-*T. circumcincta* IgG levels positively predicted survival to 4-months old, and anti-*T. circumcincta* IgG, IgA and total IgG positively predicted weight in August. In addition, high neonatal anti-*T. circumcincta* IgG levels relative to total IgG levels were associated with low strongyle faecal egg counts and positively predicted over-winter survival via effects on FEC.

6.2 Wider implications

One of the greatest problems faced by eco-immunology is the ability to determine appropriate measures of the immune response, particularly due to the lack of reagents for non-model species (Bradley & Jackson 2008). Commonly used markers of "immunocompetence" in birds, such as the skin-swelling response to challenge with phytohaemagglutinin (PHA), tell us little about which of the many arms of the vertebrate immune response are involved. In addition, challenge assays like PHA introduce a novel toxin or antigen to the host and therefore do not necessarily tell us about immune responses to ecologically relevant parasites and their consequences for host health and fitness (Graham *et al.* 2011; Demas *et al.* 2011). Studies are now calling for the use of multivariate measures of immune function to relevant parasites, rather than general immunocompetence measures (Pedersen & Babayan 2011). Here, I used measures of anti-*T. circumcincta* antibodies as they appear to be cross-reactive to a number of helminth species infecting Soay sheep (see Introduction). Strongyle nematode worm burden is known to have detrimental effects on host health and fitness (Gulland 1992; Hayward *et al.* 2011), and anti-*T. circumcincta* antibodies

have been associated with protection against *T. circumcincta* in domestic sheep (Stear *et al.* 1995; Strain *et al.* 2002). In addition, previous work in the Soay sheep have noted that these measures are associated with health and fitness (Hayward *et al.* 2014; Nussey *et al.* 2014; Watson *et al.* 2016). In this thesis I have provided a rare example of a study measuring immune responses to ecologically relevant parasites across the lifetime of individuals and linked immunity to health and fitness. I have shown that these anti-helminth antibody levels are associated with parasite burden and fitness, but these associations are dependent on antibody isotype, age and sex. Along with previous studies documenting that selection is dependent on the immune trait measured (Gonzalez *et al.* 1999; Råberg & Stjernman 2003; Parejo & Silva 2009; Nussey *et al.* 2014), these results further stress the need for a multivariate approach to understand the causes and consequences of variation in immunity in the wild.

Emerging population immunology studies in humans are investigating the causes of variation in immune traits across large cohorts (Liston *et al.* 2016) and have documented that immune phenotypes are stable across time within individuals (Tsang *et al.* 2014; Carr *et al.* 2016). However, these studies are only examining variation in an individual's immune responses across months to years, and not across the lifetime of individuals (Liston *et al.* 2016). In addition, the difficulties in repeatedly capturing individuals in natural populations across their lifetimes means there are few estimates of repeatability of immune responses in the wild (Hayward *et al.* 2014; Arriero *et al.* 2017). The results in this thesis have shown that anti-helminth antibody levels are, in general, very stable across adulthood in a relatively long-lived wild mammal. This is despite the fact that strongyle FEC varies with age and sex, as well as spatially and temporally, and has low individual repeatability (Wilson *et al.* 2004; Hayward *et al.* 2011, 2014). Therefore, despite varying exposure levels, as well as differences in food availability and weather experienced by individuals across their lifetime, individuals are very consistent in their production of anti-Tc antibodies. This is in accordance with studies in humans that have shown short-term stability in immune responses (Liston *et al.* 2016), while in dairy cows repeatability of immune traits varied across immune measures (Banos *et al.* 2013; Denholm *et al.* 2017). In comparison, in a population of wild voles, gene expression levels of candidate immune markers (IFNγ, GATA3, IL-10) were investigated, of which only IFNγ was significantly repeatable and this repeatability was low (Arriero *et al.* 2017). In the Soay sheep, the high repeatability of anti-helminth antibody responses were in part due to high genetic variation. It would be interesting to see how

repeatable other immune phenotypes, including those associated with acute infectious pathogens, are in this population or whether the high repeatability and heritability of these immune responses are due to the chronic nature of helminth infection.

Understanding the genetic basis of quantitative traits is crucial for understanding their evolution, and only in wild systems can we investigate how genetic variation in traits is maintained in the face of natural selection. The ability to determine the genetic basis of variation in immune responses in the wild is rare due to the lack of genomic resources and pedigree information for non-model species (Bradley & Jackson 2008). This study represents one of the first complete quantitative genetic breakdowns and the first genome wide association study of an immune measure in a wild system. There are few studies that have estimated the heritability of immune measures in the wild through an animal model approach (Pitala *et al.* 2007; Graham *et al.* 2010; Kim *et al.* 2013; Hayward *et al.* 2014; Sakaluk *et al.* 2014), with only one study calculating the heritability of parasite-specific immune measures to an ecologically relevant parasite (Hayward *et al.* 2014). The heritability of anti-helminth antibody levels documented in this thesis shows that there is potential for an evolutionary response to selection on parasite-specific antibody responses in this population, which is expected with the negative effects of strongyle infection on host health and fitness (Gulland 1992; Craig *et al.* 2008; Hayward *et al.* 2011). Furthermore, the heritability of anti-helminth antibody levels are considerably higher than the heritability of strongyle FEC (Coltman *et al.* 2001a; Beraldi *et al.* 2007; Brown *et al.* 2013). This has also been observed in domestic sheep, and with the methodological inaccuracies of FEC counts and imperfect association with parasite burden, parasite-specific IgA levels have been suggested as an alternative marker of breeding for resistance (Stear *et al.* 2009). Mathematical models of sheep infection dynamics with *T. circumcincta* have suggested that a quicker response in terms of improving parasite resistance is seen if selection occurs on IgA rather than FEC (Prada Jiménez de Cisneros *et al.* 2014).

Heritability, however, does not identify genetic architecture and the genes or genomic regions contributing to genetic variance in a trait. While other studies in the wild have used a candidate gene approach to identify genetic variants associated with immune responses (Turner *et al.* 2011; Brown *et al.* 2013), here I used a genome wide association study to identify SNPs associated with an immune measure in a wild population. The region identified on chromosome 24 influencing anti-*T. circumcincta* IgA levels in Chapter 2 has

not been previously identified in domestic sheep and could be of relevance to veterinary research due to the positive associations between anti-*T. circumcincta* IgA levels and parasite resistance in domestic sheep (Stear *et al.* 1995; Strain *et al.* 2002). To the best of my knowledge, only two GWAS studies using the ovine 50K SNP array have previously been carried out on anti-*T. circumcincta* IgA levels in domestic sheep (Riggio *et al.* 2013; Atlija *et al.* 2016). The study in Scottish Blackface lambs found no SNPs meeting suggestive significance (Riggio *et al.* 2013), while the other study in Spanish Churra ewes only found a genome wide significant SNP on chromosome 12 (Atlija *et al.* 2016). If the region on chromosome 24 is not associated with IgA in modern domestic breeds it would be interesting to determine the reason behind this by comparing allele frequencies between Soay sheep and domestic breeds in this region to see whether selective breeding has altered or fixed the diversity of this loci. The identification of this QTL may also be due to a genotype-by-environment effect that may only be manifested under natural conditions or could have been introduced with a historical admixture event with the Dunface breed (Feulner *et al.* 2013). The presence of a QTL with a large effect is particularly surprising considering the negative effect of parasites on host health and fitness (Schmid-Hempel 2011), which is expected to erode all genetic variation related to parasite resistance in the wild (Fisher 1930). It could be that selection on IgA is weak, as expecting from the lack of associations between IgA and survival or breeding success in Chapter 3, or could be due to undetected balancing selection mechanisms which have previously been observed in the Soay sheep for other traits (Graham *et al.* 2010; Johnston *et al.* 2013).

There were age dependent associations between strongyle faecal egg counts: lambs with low egg counts had high IgA levels, while adults with low egg counts had high IgG levels. These negative associations are in accordance with the idea that these parasite-specific immune measures are not simply a marker of exposure of the sheep to parasites, but instead may indicate protective immune responses. This also points to age-related changes in the development of immunity of helminths, which has previously been acknowledged in *T. circumcincta* infection in domestic ruminants. Protective immunity develops in two steps, the first is focused on suppressing worm growth and fecundity with IgA levels and the second involves hypersensitive reactions that lead to parasite expulsion (McNeilly *et al.* 2009). Compared to other gastrointestinal helminth infections in ruminants, protective immunity to *T. circumcincta* is much slower to develop (McRae *et al.* 2015). Research into antibody mediated resistance to *T. circumcincta* in domestic sheep has mainly focussed on

parasite-specific IgA, and to a lesser extent IgE, levels following earlier research showing associations between this isotype and female worm length and fecundity (Stear *et al.* 1995; Strain *et al.* 2002). Veterinary research has also typically focussed on lambs and not adults (Atlija *et al.* 2016). Here I have shown that IgG may be an important isotype in *T. circumcincta* infection in older animals. IgG has previously been associated with protection against helminths in mice (Appleton & McGregor 1987; Blackwell & Else 2001; Harris *et al.* 2006; McCoy *et al.* 2008), and has been associated with reduced FEC or worm burden for adult ruminants (Williams *et al.* 2010; McBean *et al.* 2016). In addition, complement, which can be activated by IgG, has also been associated with protection against helminths in ruminants (Li *et al.* 2010; Guo *et al.* 2016). If IgG is also associated with parasite resistance in adult domestic sheep, this could have important implications for the domestic sheep industry. For instance, selective breeding for parasite resistance in ewes could reduce the use of anthelmintics and reduce livestock turnover (Benavides *et al.* 2016). In addition, ewes have the potential to contribute massively to faecal egg counts during the periparturient rise following parturition, thereby affecting the exposure of lambs to parasites (McRae *et al.* 2015). Potential areas of future investigation would be to determine the mechanism by which IgG is mediating a reduction in strongyle faecal egg counts, and whether this is due to complement as has been previously implicated (Li *et al.* 2010; Guo *et al.* 2016).

Studies in mice have documented that maternal antibody transfer of parasite-specific antibodies is associated with protection of neonates against helminth infection (Appleton & McGregor 1987; Harris *et al.* 2006), but studies in the wild documenting protection of neonates against infection is rare (Kallio *et al.* 2006). In wild bird populations, the role of maternal antibodies has typically involved immunisation of mothers (Grindstaff *et al.* 2006; Staszewski *et al.* 2007) or measurement of total antibodies transferred (Pihlaja *et al.* 2006; Moreno *et al.* 2008). Consequently, few studies are able to tell us about the potential for passive transfer of protection to relevant micro- or macroparasites that the neonate is exposed to in early life (but see Kallio *et al.* 2006; Gasparini *et al.* 2009). In livestock, studies have generally focussed on total IgG levels, and consequently it is unclear whether parasite-specific antibody levels are associated with offspring growth and fitness (Pfeffer *et al.* 2005). The benefits of studying maternally transferred antibody levels in the Soay sheep are due to complete lack of immune transfer prenatally, the slow development of immune competence in lambs and the non-specific uptake of antibodies which allow these antibodies to be a general reflection of levels ingested in the colostrum (Brambell 1970; Butler 1999).

In this thesis, the measurement of both parasite-specific antibody levels and total antibody levels allowed the investigation of the fitness consequences of antibody responses to an ecologically relevant parasite, alongside an index of total protein provided in milk by the mother. I found that anti-*T. circumcincta* IgG levels were principally associated with offspring growth and survival, and this again highlights the importance of this isotype in this study system. Crucially, I found evidence for direct protection of neonatal anti-*T. circumcincta* IgG levels against helminths, and this association drove long-term fitness benefits through effects on parasite resistance. In comparison, total IgG was only associated with improved growth of offspring, providing evidence for the importance of maternally-transferred parasite-specific responses for long-term health and fitness rather than total antibodies transferred. In domestic lambs on pasture, the levels of parasite-specific IgG in colostrum that is subsequently transferred to offspring may improve health of lambs in early life. Furthermore, numerous studies in birds have documented no association between maternal antibodies and growth (Grindstaff *et al.* 2006; Staszewski *et al.* 2007; Tschirren *et al.* 2009), and this may be due to the fact that these studies are not differentiating between the specificity of antibodies transferred. Although my results indicate that parasite-specific IgG levels may have direct immunoprotective effects in terms of parasite resistance, there was a weak negative correlation between neonatal and August antibody levels. It would be interesting to see whether clear blocking effects of maternal antibodies on endogenous immune responses in early life can be determined. Such blocking effects have been observed in humans (Siegrist 2003) but evidence is rare in wild systems (Staszewski *et al.* 2007).

The quantification of variance in maternally transferred antibody levels attributable to individual mothers has not previously been reported in the wild, although it has been noted that female birds are consistent in maternal antibody transfer (Gasparini *et al.* 2001; Coakley *et al.* 2014). Since the anti-helminth antibody levels measured here likely originate from the gut and are passively transferred into colostrum, the consistency of mothers in maternal antibody transfer may be due to the general high repeatability observed in plasma anti-helminth antibody levels in adults (Pastoret *et al.* 1998; Butler 1999). It would be interesting to compare the size of the maternal effects and maternal genetic effects for total antibody levels, since all IgG but only 40% of IgA in the colostrum is from the bloodstream, the rest being produced locally in the mammary gland (Tizard 2012). Furthermore, a breakdown of the variance in maternally transferred antibody levels between maternal genetic, maternal environment and additive genetic components has not been attempted in a wild population

(Boulinier & Staszewski 2008). The lack of an additive genetic effect may be due to selection eroding all additive genetic variance due to the effects on offspring fitness, while maternal genetic variance is maintained by the lack of clear maternal costs. The presence of a significant maternal genetic, but not an additive genetic, component will have strong impacts on the evolutionary potential of the trait since an evolutionary response can only come about via selection on the mother (Räsänen & Kruuk 2007). A study in piglets suggested a similar partitioning of variance may also be seen in livestock and found that maternal genetic effects explained four times the variance in neonatal immunocrit levels compared to additive genetic effects (Rohrer *et al.* 2014). The economic cost of failure of passive immune transfer is considerable (Raboisson *et al.* 2016). If maternal genetic effects explain considerable variance in maternal antibody transfer in livestock, this suggests that attempts to identify genetic variants associated with passive immune failure for selective breeding purposes should look at genetic variants of the mother rather than the offspring which has been the focus so far (Laegreid *et al.* 2002; Clawson *et al.* 2004; Rohrer *et al.* 2014).

6.3 Study limitations

Although the Soay sheep on St Kilda have been subject to natural selection for thousands of years, they are an ancient breed of domestic sheep and have been subject to artificial selection in the past. In addition, in comparison to other wild animals, it is a simple island community with no predators (Clutton-Brock & Pemberton 2004). Further, while Soay sheep are infected with numerous helminth parasites, there is a species-poor microparasite community, with few viral and bacterial pathogens that are common in domestic Scottish sheep (Graham *et al.* 2016). Consequently, while the Soay sheep exhibit much higher genetic diversity and a more variable environment than the laboratory models where immunity is typically studied, they may be an oversimplification of a complex system typically seen in the wild.

Some methodological limitations are posed by the non-invasive and isolated nature of the study system. For instance, blood and faecal samples of the entire population are only routinely collected yearly, and therefore we do not know how anti-helminth antibodies or FEC vary within years. In addition, I have used FEC as a measure of strongyle worm burden,

which measures egg output rather than actual worm burden. Although there is a correlation between worm burden and FEC in the Soay sheep (Grenfell *et al.* 1995), FEC has notably methodological limitations and can be highly variable. For instance, individuals may be infected but have no egg output, in the cases of individuals harbouring immature worms or single-sex infections. Further, negative density-dependence means that there is typically a non-linear relationship between worm burden and female egg output (Stear & Bishop 1999; Bishop & Stear 2000).

In this study, I have only measured one aspect of anti-helminth immunity in the form of anti-parasite antibodies. However, this is an oversimplification of both the immune system and the complex immune response to helminth parasites observed in domestic sheep (McRae *et al.* 2015). Further, there are difficulties in determining whether these antibody levels are a protective response or are simply a measure of parasite exposure. Although I found negative associations between these antibody isotypes and FEC suggesting a protective role of these antibodies it is possible that antibody levels are in part reflecting parasite exposure. In addition, the associations between anti-helminth antibody isotypes and strongyle FEC are typically weak, suggesting that there are numerous other factors beyond antibodies that influence strongyle faecal egg count which were beyond the aims and scope of this thesis.

6.4 Further work

Determining the amount of additive genetic variance underlying a phenotypic trait is the first step in determining the trait's evolutionary potential. The evolutionary potential of traits are also dependent on genetic correlations with other traits which may be masked at the phenotypic level (Kruuk *et al.* 2008). Genetic correlations may come about due to genes in high linkage disequilibrium or pleiotropy and may impose evolutionary constraints (Lande 1982). Estimates of genetic correlations, in addition to other environmental covariances, can be estimated from multivariate analyses (Blows 2007). Genetic correlations between immune and other traits are evident from selection lines of domestic animals: calves that were selected for early growth had lower IgG of colostral origin (Bradley *et al.* 1979; Muggli *et al.* 1984), while chickens selected for high humoral immune response had reduced thymus weights, as well as reduced fertility and reproduction (Ubosi, Gross & Siegel 1985; Martin *et al.* 1990; Siegel, Gross & Cherry 2009). In this thesis, I did not estimate genetic correlations

among immune and other traits but as a future step in analyses, I could look at genetic correlations across both maternally transferred neonatal and endogenously produced anti-helminth antibody isotypes. For instance, a positive genetic correlation between neonatal total and parasite-specific antibody levels might be expected, but this could be higher for IgG compared to IgA since the majority of IgA in the colostrum is locally produced (Tizard 2012). In addition, I could look at maternal genetic correlations between maternal antibody levels and other traits of the mother (such as her endogenous antibody levels, weight and strongyle faecal egg counts) as well as other offspring traits (birth weight). For endogenous antibodies, it would be interesting to see whether there were significant genetic correlations with weight and strongyle FEC. These multivariate models will provide us with more information about the evolutionary potential of anti-helminth immune responses, since selection will not act on traits in isolation, but will act on a number of traits simultaneously (Walsh & Blows 2009).

Some analyses were outside the scope of the quantitative genetic analyses in Chapter 2 but would add to our knowledge of the genetic architecture underlying endogenous anti-parasite immune responses. In order to maximise the use of all of the data, it may be possible to run the GWAS with all repeat measures using repeatABEL (Rönnegård *et al.* 2016) or in an animal model framework (Johnston *et al.* 2016). In addition, regional heritability analyses could help identify rare and small effect variants that may be associated with anti-*T. circumcincta* IgA, IgE and IgG levels by looking at the proportion of variance explained by defined regions of the genome (following Bérénos *et al.* 2015; Johnston *et al.* 2016). Using this approach, it would be possible to calculate the proportion of additive genetic variance explained by the 5 Mb region associated with anti-*T. circumcincta* IgA levels, rather than individual SNPs alone. Although I found no associations between these SNPs and fitness measures, another way to investigate selection on this region would be using gene-drop simulations. Gene-drop analyses would be able to investigate whether any changes in allele frequencies of SNPs in this region between 1985-present are due to stochastic processes (i.e. genetic drift) or may be due to selection. Finally, although I had a potential candidate gene in this region, CLEC16A, I was unable to fully confirm the association with this gene. To do so would require sequence information to provide details of potential causal variants in this region, while expression data would allow the investigation of whether these genetic variants affect CLEC16A gene or protein expression.

In addition to running a GWAS on endogenous anti-parasite antibody levels, it would also be possible to test whether any SNPs were associated with maternally-derived neonatal antibody levels and see if these were consistent with SNPs associated with endogenous antibody levels as expected if they are partly of bloodstream origin. This would be a first for a wild system, with only a handful of studies investigated genetic variants underlying passive immune transfer in domestic ruminants (Laegreid *et al.* 2002; Clawson *et al.* 2004; Rohrer *et al.* 2014). Rather than looking at genetic variants of the offspring as most of these studies have done, the lack of any direct additive genetic variance underlying neonatal antibody levels would suggest that any GWAS should be run as a maternal trait. A preliminary GWAS of neonatal anti-*T. circumcincta* IgA, IgE and IgG antibody levels run as a maternal trait found that IgA levels were associated with a region on chromosome 12, and a single SNP on chromosome 18 and 24 each (Figure 6.1). This region on chromosome 12 contains a number of immune genes including a candidate gene in PIGR, encoding the pIgR receptor that transports primarily IgA but also IgM across epithelial cells including the mammary gland (Kacskovics 2004). In the sheep mammary gland, pIgR expression is upregulated a few weeks before parturition and peaks late in lactation, potentially due to the combined effects of hormones (e.g. glucocorticoids) and cytokines (IFNγ) (Rincheval-Arnold, Belair & Djiane 2002b; Rincheval-Arnold *et al.* 2002a). In addition, the hit on chromosome 24 was the top hit for endogenous anti-*T. circumcincta* IgA levels. Further work will be needed to confirm this association, and investigate how much variation in the trait these SNPs and this QTL on chromosome 12 explains.

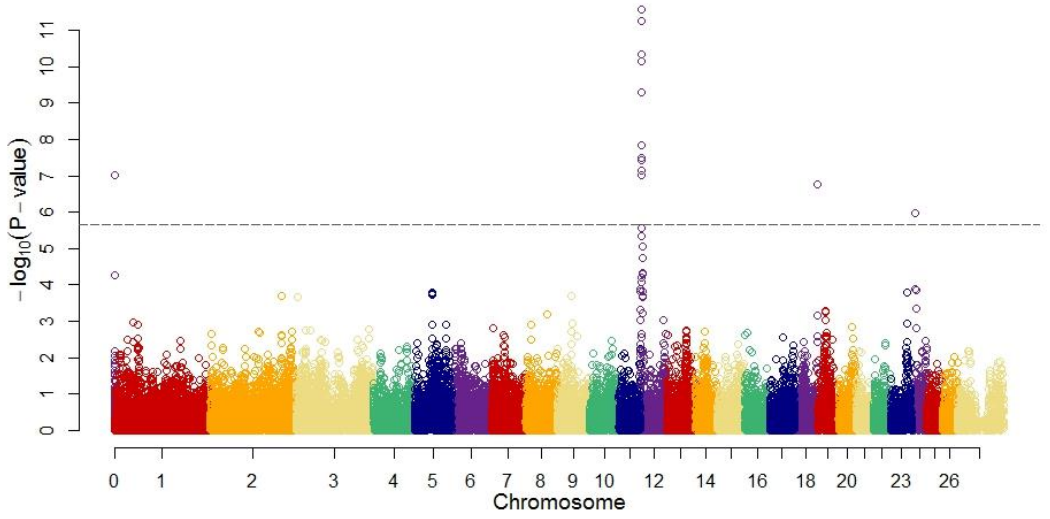

Figure 6.1. Genome-wide association results for neonatal anti-*T. circumcincta* IgA levels measured as a maternal trait in wild Soay sheep using the average levels in her offspring (excluding twins) corrected for capture age. The Manhattan plot of p-values is corrected for inflation (lambda) and the dashed line indicates the Bonferroni genome-wide significance threshold.

Although this thesis has focussed on the genetic basis of parasite-specific immune responses, there is considerable information on the Soay sheep for quantifying environmental factors associated with immune responses. In particular, the availability of individual ranging behaviour and foliage cover across the study area allows for the quantification of home range quality, size and overlap (Regan *et al.* 2016, 2017). It has been observed that forage availability and quality vary markedly across the study area and this variation is associated with male and female lifetime reproductive success (Regan *et al.* 2016). It would be interesting to see how forage quality may affect immune responses, particularly in the light of considerable permanent environment effects underlying variance in these immune traits. It might be expected that individuals with good quality home ranges in terms of forage quality would be in better condition and therefore have higher antibody levels (Regan *et al.* 2016). Studies from humans have found that cohabitation results in greater similarity in levels of cellular components of the immune system, and it would be interesting to see whether we could detect the same convergence of immune phenotypes with home range overlap in the Soay sheep (Carr *et al.* 2016). The quality of a mother's home range may also impact maternal antibody transfer and it might be predicted that mothers in good quality home

ranges would transfer higher maternal antibodies. In addition, studying how spatial patterns of anti-parasite immune responses vary with spatial patterns of strongyle larvae pasture counts could provide us with more information on the relationship between parasite exposure and immune responses.

Although I have measured immune responses in April and August, additional blood samples of males are also taken during the rut in October – December (Preston *et al.* 2012). It would be interesting to investigate the causes and consequences of variation in immune responses at this stage. During the rut males compete for females and spend significantly less time foraging (Clutton-Brock & Pemberton 2004), and therefore there may be trade-offs between reproductive effort and immunity (Sheldon & Verhulst 1996). These rut samples are also the closest blood samples we have to winter, and may be the best indication of the state of males going into the toughest part of the year (Clutton-Brock & Pemberton 2004). While I found an association between August anti-*T. circumcincta* IgG levels and over-winter survival in females, this was not observed in males. It may be that August measures are not as good a reflection of the state of males going into winter as they are for females, and these rut samples may prove to be a better indicator.

6.5 Concluding remarks

The Soay sheep population on St Kilda offer an excellent opportunity to study immunity in the wild due to the availability of immune reagents and knowledge of protective immune responses from livestock, the availability of pedigree and genomic data, as well as the comprehensive life history data available for individuals in the study population. In this thesis I present analyses of two novel datasets on immunity in a wild study system. The first dataset measures anti-helminth antibody levels of multiple isotypes to an ecologically relevant parasite over a 25 year period, thereby producing a unique longitudinal dataset that provides information on variation in immunity over the lifetimes of individuals. The second dataset measures maternally-derived anti-helminth antibody levels in neonates over the same large timescale and was able to differentiate fitness benefits of total protein delivery to parasite-specific measures. I found that mothers were repeatable in the level of anti-helminth antibody levels transferred to neonates and there were considerable maternal genetic effects underlying these traits. In comparison, endogenously-produced antibody levels were highly

repeatable across lifetimes, and there was considerable additive genetic variance underlying these traits. In early life, I found that both maternally-derived and endogenous antibodies are associated with resistance to strongyles, while maternally-derived antibodies were important for early growth and survival, as well as overwinter survival via potential immunoprotective effects against strongyle worms. In comparison, fitness benefits in adulthood were associated with higher endogenous anti-helminth antibody levels. Both these datasets illustrate that maternal effects and genetic variation can have strong effects on variation in immunity in the wild, and this variation in turn can have health and fitness consequences for individuals. Both the datasets used in this thesis allow a myriad of possibilities for future research with the potential to advance our understanding of the maintenance of variation in immunity and associations between immunity and fitness in nature.

Bibliography

Abolins, S., King, E.C., Lazarou, L., Weldon, L., Hughes, L., Drescher, P., Raynes, J.G., Hafalla, J.C.R., Viney, M.E. & Riley, E.M. (2017) The comparative immunology of wild and laboratory mice, *Mus musculus domesticus*. *Nature Communications*, **8**, 14811.

Abolins, S.R., Pocock, M.J.O., Hafalla, J.C.R., Riley, E.M. & Viney, M.E. (2011) Measures of immune function of wild mice, *Mus musculus*. *Molecular Ecology*, **20**, 881–892.

Acevedo-Whitehouse, K. & Cunningham, A.A. (2006) Is MHC enough for understanding wildlife immunogenetics? *Trends in Ecology & Evolution*, **21**, 433–438.

Agrawal, A.A., Laforsch, C. & Tollrian, R. (1999) Transgenerational induction of defences in animals and plants. *Nature*, **401**, 60–63.

Alberts, B., Johnson, A., Lewis, J., Morgan, D., Raff, M., Roberts, K. & Walter, P. (2015) *Molecular Biology of the Cell*, 6th Ed. Garland Science, New York.

Albrecht, P., Ennis, F.A., Saltzman, E.J. & Krugman, S. (1977) Persistence of maternal antibody in infants beyond 12 months: mechanism of measles vaccine failure. *The Journal of Pediatrics*, **91**, 715–718.

Allen, J.E. & Maizels, R.M. (2011) Diversity and dialogue in immunity to helminths. *Nature Reviews Immunology*, **11**, 375–388.

Altizer, S., Dobson, A., Hosseini, P., Hudson, P., Pascual, M. & Rohani, P. (2006) Seasonality and the dynamics of infectious diseases. *Ecology Letters*, **9**, 467–484.

Anderson, R.W. (1995) On the maternal transmission of immunity: a "molecular attention" hypothesis. *BioSystems*, **34**, 87–105.

Anderson, R.M. & Gordon, D.M. (1982) Processes influencing the distribution of parasite numbers within host populations with special emphasis on parasite-induced host mortalities. *Parasitology*, **85**, 373–398.

Anderson, R.M. & May, R.M. (1978) Regulation and stability of host-parasite population interactions: I. Regulatory processes. *Journal of Animal Ecology*, **47**, 219–247.

Anderson, R.M. & May, R.M. (1985) Helminth infections of humans: mathematical models, population dynamics, and control. *Advances in Parasitology*, **24**, 1–101.

Anthony, R.M., Rutitzky, L.I., Urban, J.F., Stadecker, M.J., Gause, W.C. & Gause, W.C. (2007) Protective immune mechanisms in helminth infection. *Nature Reviews Immunology*, **7**, 975–987.

Appleton, J.A. & McGregor, D.D. (1987) Characterization of the immune mediator of rapid expulsion of *Trichinella spiralis* in suckling rats. *Immunology*, **62**, 477–484.

Argüello, A., Castro, N., Álvarez, S. & Capote, J. (2006) Effects of the number of lactations and litter size on chemical composition and physical characteristics of goat colostrum. *Small Ruminant Research*, **64**, 53–59.

Argüello, A., Castro, N., Zamorano, M.J., Castroalonso, A. & Capote, J. (2004) Passive

transfer of immunity in kid goats fed refrigerated and frozen goat colostrum and commercial sheep colostrum. *Small Ruminant Research*, **54**, 237–241.

Arriero, E., Wanelik, K.M., Birtles, R.J., Bradley, J.E., Jackson, J.A., Paterson, S. & Begon, M. (2017) From the animal house to the field: are there consistent individual differences in immunological profile in wild populations of field voles (*Microtus agrestis*)? (ed MC Fisher). *PLoS ONE*, **12**, e0183450.

Atlija, M., Arranz, J.-J., Martinez-Valladares, M. & Gutiérrez-Gil, B. (2016) Detection and replication of QTL underlying resistance to gastrointestinal nematodes in adult sheep using the ovine 50K SNP array. *Genetics Selection Evolution*, **48**, 4.

Aulchenko, Y.S., Ripke, S., Isaacs, A. & van Duijn, C.M. (2007) GenABEL: an R library for genome-wide association analysis. *Bioinformatics*, **23**, 1294–1296.

Banos, G., Wall, E., Coffey, M.P., Bagnall, A., Gillespie, S., Russell, G.C. & McNeilly, T.N. (2013) Identification of immune traits correlated with dairy cow health, reproduction and productivity. *PLoS ONE*, **8**, e65766.

Barson, N.J., Aykanat, T., Hindar, K., Baranski, M., Bolstad, G.H., Fiske, P., Jacq, C., Jensen, A.J., Johnston, S.E., Karlsson, S., Kent, M., Moen, T., Niemelä, E., Nome, T., Næsje, T.F., Orell, P., Romakkaniemi, A., Sægrov, H., Urdal, K., Erkinaro, J., Lien, S. & Primmer, C.R. (2015) Sex-dependent dominance at a single locus maintains variation in age at maturity in salmon. *Nature*, **528**, 405–408.

Bates, D., Mächler, M., Bolker, B.M. & Walker, S.C. (2015) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, **67**, 1–48.

Beavis, W. (1994) The power and deceit of QTL experiments: lessons from comparative QTL studies. *Proceedings of the Forty-Ninth Annual Corn and Sorghum Industry Research Conference*, pp. 250–266. American Seed Trade Association, Washington.

Benavides, M.V., Sonstegard, T.S. & Van Tassell, C. (2016) Genomic regions associated with sheep resistance to gastrointestinal nematodes. *Trends in Parasitology*, **32**, 470–480.

Beraldi, D., McRae, A.F., Gratten, J., Pilkington, J.G., Slate, J., Visscher, P.M. & Pemberton, J.M. (2007) Quantitative trait loci (QTL) mapping of resistance to strongyles and coccidia in the free-living Soay sheep (*Ovis aries*). *International Journal for Parasitology*, **37**, 121–129.

Bérénos, C., Ellis, P.A., Pilkington, J.G., Lee, S.H., Gratten, J. & Pemberton, J.M. (2015) Heterogeneity of genetic architecture of body size traits in a free-living population. *Molecular Ecology*, **24**, 1810–1830.

Berman, M., Gaillard, J.-M. & Weimerskirch, H. (2009) Contrasted patterns of age-specific reproduction in long-lived seabirds. *Proceedings of the Royal Society of London B: Biological Sciences*, **276**, 375–382.

Bernardo, J. (1996) Maternal effects in animal ecology. *American Zoologist*, **36**, 83–105.

Beura, L.K., Hamilton, S.E., Bi, K., Schenkel, J.M., Odumade, O.A., Casey, K.A., Thompson, E.A., Fraser, K.A., Rosato, P.C., Filali-Mouhim, A., Sekaly, R.P., Jenkins, M.K., Vezys, V., Haining, W.N., Jameson, S.C. & Masopust, D. (2016) Normalizing the environment recapitulates adult human immune traits in laboratory mice. *Nature*, **532**, 512–516.

Bielmann, V., Gillan, J., Perkins, N.R., Skidmore, A.L., Godden, S. & Leslie, K.E. (2010)

An evaluation of Brix refractometry instruments for measurement of colostrum quality in dairy cattle. *Journal of Dairy Science*, **93**, 3713–3721.

Bishop, S.C., Jackson, F., Coop, R.L. & Stear, M.J. (2004) Genetic parameters for resistance to nematode infections in Texel lambs and their utility in breeding programmes. *Animal Science*, **78**, 185–194.

Bishop, S.C. & Stear, M.J. (2000) The use of a gamma-type function to assess the relationship between the number of adult *Teladorsagia circumcincta* and total egg output. *Parasitology*, **121**, 435–440.

Bishop, S.C. & Stear, M.J. (2001) Inheritance of faecal egg counts during early lactation in Scottish Blackface ewes facing mixed, natural nematode infections. *Animal Science*, **73**, 389–395.

Björkholm, B., Granström, M., Taranger, J., Wahl, M. & Hagberg, L. (1995) Influence of high titers of maternal antibody on the serologic response of infants to diphtheria vaccination at three, five and twelve months of age. *The Pediatric Infectious Disease Journal*, **14**, 846–850.

Blackwell, N.M. & Else, K.J. (2001) B cells and antibodies are required for resistance to the parasitic gastrointestinal nematode *Trichuris muris*. *Infection and Immunity*, **69**, 3860–3868.

Blecha, F., Bull, R.C., Olson, D.P., Ross, R.H. & Curtis, S. (1981) Effects of prepartum protein restriction in the beef cow on immunoglobin content in blood and colostral whey and subsequent immunoglobin absorption by the neonatal calf. *Journal of Animal Science*, **53**, 1174–1180.

Blows, M.W. (2007) A tale of two matrices: multivariate approaches in evolutionary biology. *Journal of Evolutionary Biology*, **20**, 1–8.

Blum, J.W. & Hammon, H. (2000) Colostrum effects on the gastrointestinal tract, and on nutritional, endocrine and metabolic parameters in neonatal calves. *Livestock Production Science*, **66**, 151–159.

Bonneaud, C., Mazuc, J., Gonzalez, G., Haussy, C., Chastel, O., Faivre, B. & Sorci, G. (2003) Assessing the cost of mounting an immune response. *The American Naturalist*, **161**, 367–379.

Bonneaud, C., Sinsheimer, J.S., Richard, M., Chastel, O. & Sorci, G. (2009) MHC polymorphisms fail to explain the heritability of phytohaemagglutinin-induced skin swelling in a wild passerine. *Biology Letters*, **5**, 784–787.

Bouix, J., Krupinski, J., Rzepecki, R., Nowosad, B., Skrzyzala, I., Roborzynski, M., Fudalewicz-Niemczyk, W., Skalska, M., Malczewski, A. & Gruner, L. (1998) Genetic resistance to gastrointestinal nematode parasites in Polish long-wool sheep. *International Journal for Parasitology*, **28**, 1797–1804.

Boulinier, T. & Staszewski, V. (2008) Maternal transfer of antibodies: raising immuno-ecology issues. *Trends in Ecology & Evolution*, **23**, 282–288.

Bradley, J.E. & Jackson, J.A. (2008) Measuring immune system variation to help understand host-pathogen community dynamics. *Parasitology*, **135**, 807–823.

Bradley, J.A., Niilo, L. & Dorward, W.J. (1979) Some observations on serum gammaglobulin concentrations in suckled beef calves. *Canadian Veterinary Journal*, **20**, 227–232.

Brambell, F.W.R. (1970) *The Transmission of Passive Immunity from Mother to Young (Frontiers of Biology Volume 18)* (eds A Neuberger and EL Tatum). Elsevier, New York.

Britti, D., Massimini, G., Peli, A., Luciani, A. & Boari, A. (2005) Evaluation of serum enzyme activities as predictors of passive transfer status in lambs. *Journal of the American Veterinary Medical Association*, **226**, 951–955.

Brodin, P., Jojic, V., Gao, T., Bhattacharya, S., Angel, C.J.L., Furman, D., Shen-Orr, S., Dekker, C.L., Swan, G.E., Butte, A.J., Maecker, H.T. & Davis, M.M. (2015) Variation in the human immune system is largely driven by non-heritable influences. *Cell*, **160**, 37–47.

Bronson, P.G., Chang, D., Bhangale, T., Seldin, M.F., Ortmann, W., Ferreira, R.C., Urcelay, E., Pereira, L.F., Martin, J., Plebani, A., Lougaris, V., Friman, V., Freiberger, T., Litzman, J., Thon, V., Pan-Hammarstrom, Q., Hammarstrom, L., Graham, R.R., Behrens, T.W., Pan-Hammarström, Q., Hammarström, L., Graham, R.R. & Behrens, T.W. (2016) Common variants at PVT1, ATG13-AMBRA1, AHI1 and CLEC16A are associated with selective IgA deficiency. *Nature Genetics*, **48**, 1425–1429.

Brown, E.A., Pilkington, J.G., Nussey, D.H., Watt, K.A., Hayward, A.D., Tucker, R., Graham, A.L., Paterson, S., Beraldi, D., Pemberton, J.M. & Slate, J. (2013) Detecting genes for variation in parasite burden and immunological traits in a wild population: testing the candidate gene approach. *Molecular Ecology*, **22**, 757–773.

Buechler, K., Fitze, P.S., Gottstein, B., Jacot, A. & Richner, H. (2002) Parasite-induced maternal response in a natural bird population. *Journal of Animal Ecology*, **71**, 247–252.

Butler, J.E. (1999) Immunoglobulins and immunocytes in animal milks. *Mucosal Immunology*, 2nd Ed. (eds P.L. Ogra, J. Mestecky, M.E. Lamm, W. Strober, J. Bienenstock & J.R. McGhee), pp. 1531–1554. Academic Press, New York.

Butler, D.G., Cullis, B.R., Gilmour, A.R. & Gogel, B.J. (2009) *Mixed Models for S Language Environments, ASReml-R Reference Manual*. Queensland Department of Primary Industries and Fisheries, Brisbane.

Byers, D.L., Platenkamp, G.A.J. & Shaw, R.G. (1997) Variation in seed characters in *Nemophila menziesii*: evidence of a genetic basis for maternal effect. *Evolution*, **51**, 1445–1456.

Cáceres, V.M., Strebel, P.M. & Sutter, R.W. (2000) Factors determining prevalence of maternal antibody to measles virus throughout infancy: a review. *Clinical Infectious Diseases*, **31**, 110–119.

Calsbeek, R., Bonneaud, C. & Smith, T.B. (2008) Differential fitness effects of immunocompetence and neighbourhood density in alternative female lizard morphs. *Journal of Animal Ecology*, **77**, 103–109.

Campbell, S.G., Siegel, M.J. & Knowlton, B.J. (1977) Sheep immunoglobulins and their transmission to the neonatal lamb. *New Zealand Veterinary Journal*, **25**, 361–365.

Carr, E.J., Dooley, J., Garcia-Perez, J.E., Lagou, V., Lee, J.C., Wouters, C., Meyts, I., Goris, A., Boeckxstaens, G., Linterman, M.A. & Liston, A. (2016) The cellular composition of the human immune system is shaped by age and cohabitation. *Nature Immunology*, **17**, 461–468.

Castro, N., Capote, J., Morales-Delanuez, A., Rodríguez, C. & Argüello, A. (2009) Effects of newborn characteristics and length of colostrum feeding period on passive immune transfer in goat kids. *Journal of Dairy Science*, **92**, 1616–1619.

Catchpole, E.A., Morgan, B.J.T., Coulson, T.N., Freeman, S.N. & Albon, S.D. (2000) Factors influencing Soay sheep survival. *Journal of the Royal Statistical Society: Series C (Applied Statistics)*, **49**, 453–472.

Christe, P., De Lope, F., González, G., Saino, N. & Møller, A.P. (2001) The influence of environmental conditions on immune responses, morphology and recapture probability of nestling house martins (*Delichon urbica*). *Oecologia*, **126**, 333–338.

Christe, P., Møller, A.P. & de Lope, F. (1998) Immunocompetence and nestling survival in the house martin: the tasty chick hypothesis. *Oikos*, **83**, 175–179.

Clapperton, M., Glass, E.J. & Bishop, S.C. (2008) Pig peripheral blood mononuclear leucocyte subsets are heritable and genetically correlated with performance. *Animal*, **2**, 1575–1584.

Clawson, M.L., Heaton, M.P., Chitko-McKown, C.G., Fox, J.M., Smith, T.P.L., Snelling, W.M., Keele, J.W. & Laegreid, W.W. (2004) Beta-2-microglobulin haplotypes in U.S. beef cattle and association with failure of passive transfer in newborn calves. *Mammalian Genome*, **15**, 227–236.

Clutton-Brock, T. & Pemberton, J. (2004) *Soay Sheep: Dynamics and Selection in an Island Population*. Cambridge University Press, Cambridge, UK.

Clutton-Brock, T.H., Price, O.F., Albon, S.D. & Jewell, P.A. (1991) Persistent Instability and Population Regulation in Soay Sheep. *The Journal of Animal Ecology*, **60**, 593.

Clutton-Brock, T.H., Price, O.F., Albon, S.D. & Jewell, P.A. (1992) Early Development and Population Fluctuations in Soay Sheep. *Journal of Animal Ecology*, **61**, 381–396.

Clutton-Brock, T.H., Stevenson, I.R., Marrow, P., MacColl, A.D., Houston, A.I. & McNamara, J.M. (1996) Population fluctuations, reproductive costs and life-history tactics in female Soay sheep. *Journal of Animal Ecology*, **65**, 675–689.

Coakley, C.M., Staszewski, V., Herborn, K.A. & Cunningham, E.J. (2014) Factors affecting the levels of protection transferred from mother to offspring following immune challenge. *Frontiers in Zoology*, **11**, 46.

Coltman, D.W., Pilkington, J., Kruuk, L.E.B., Wilson, K. & Pemberton, J.M. (2001a) Positive genetic correlation between parasite resistance and body size in a free-living ungulate population. *Evolution*, **55**, 2116–2125.

Coltman, D.W., Wilson, K., Pilkington, J.G., Stear, M.J. & Pemberton, J.M. (2001b) A microsatellite polymorphism in the gamma interferon gene is associated with resistance to gastrointestinal nematodes in a naturally-parasitized population of Soay sheep. *Parasitology*, **122**, 571–582.

Conneely, M., Berry, D.P., Sayers, R., Murphy, J.P., Lorenz, I., Doherty, M.L. & Kennedy, E. (2013) Factors associated with the concentration of immunoglobulin G in the colostrum of dairy cows. *Animal*, **7**, 1824–1832.

Coulson, T., Catchpole, E.A., Albon, S.D., Morgan, B.J., Pemberton, J.M., Clutton-Brock, T.H., Crawley, M.J. & Grenfell, B.T. (2001) Age, sex, density, winter weather, and population crashes in Soay sheep. *Science*, **292**, 1528–1531.

Coulson, T., Ezard, T.H.G., Pelletier, F., Tavecchia, G., Stenseth, N.C., Childs, D.Z., Pilkington, J.G., Pemberton, J.M., Kruuk, L.E.B., Clutton-Brock, T.H. & Crawley, M.J. (2008) Estimating the functional form for the density dependence from life history data. *Ecology*, **89**, 1661–1674.

Cox, F.E.G. (2002) History of human parasitology. *Clinical microbiology reviews*, **15**, 595–612.

Craig, B.H., Pilkington, J.G., Kruuk, L.E.B. & Pemberton, J.M. (2007) Epidemiology of parasitic protozoan infections in Soay sheep (*Ovis aries* L.) on St Kilda. *Parasitology*, **134**, 9–21.

Craig, B.H., Pilkington, J.G. & Pemberton, J.M. (2006) Gastrointestinal nematode species burdens and host mortality in a feral sheep population. *Parasitology*, **133**, 485–496.

Craig, B.H., Tempest, L.J., Pilkington, J.G. & Pemberton, J.M. (2008) Metazoan-protozoan parasite co-infections and host body weight in St Kilda Soay sheep. *Parasitology*, **135**, 433–441.

Crawford, A.M., Paterson, K.A., Dodds, K.G., Diez Tascon, C., Williamson, P.A., Roberts Thomson, M., Bisset, S.A., Beattie, A.E., Greer, G.J., Green, R.S., Wheeler, R., Shaw, R.J., Knowler, K. & McEwan, J.C. (2006) Discovery of quantitative trait loci for resistance to parasitic nematode infection in sheep: I. Analysis of outcross pedigrees. *BMC Genomics*, **7**, 178.

Crawley, M.J., Albon, S.D., Bazely, D.R., Milner, J.M., Pilkington, J.G. & Tuke, A.L. (2004) Vegetation and sheep population dynamics. *Soay Sheep: Dynamics and Selection in an Island Population* (eds T.H. Clutton-Brock & J.M. Pemberton), pp. 89–112. Cambridge University Press, Cambridge.

Crombie, J.A. & Anderson, R.M. (1985) Population dynamics of *Schistosoma mansoni* in mice repeatedly exposed to infection. *Nature*, **315**, 491–493.

Dardillat, J., Trillat, G. & Larvor, P. (1978) Colostrum immunoglobulin concentration in cows: relationship with their calf mortality and with the colostrum quality of their female offspring. *Annales de Recherches Vétérinaires*, **9**, 375–384.

Davies, G., Stear, M.J., Benothman, M., Abuagob, O., Kerr, a, Mitchell, S. & Bishop, S.C. (2006) Quantitative trait loci associated with parasitic infection in Scottish blackface sheep. *Heredity*, **96**, 252–258.

Demas, G.E. & Nelson, R.J. (2012) *Ecoimmunology*. Oxford University Press, New York.

Demas, G.E., Zysling, D.A., Beechler, B.R., Muehlenbein, M.P. & French, S.S. (2011) Beyond phytohaemagglutinin: assessing vertebrate immune function across ecological contexts. *Journal of Animal Ecology*, **80**, 710–730.

Denholm, S.J., McNeilly, T.N., Banos, G., Coffey, M.P., Russell, G.C., Bagnall, A., Mitchell, M.C. & Wall, E. (2017) Estimating genetic and phenotypic parameters of cellular immune-associated traits in dairy cows. *Journal of Dairy Science*, **100**, 2850–2862.

Dobson, A., Lafferty, K.D., Kuris, A.M., Hechinger, R.F. & Jetz, W. (2008) Homage to Linnaeus: How many parasites? How many hosts? *Proceedings of the National Academy of Sciences of the USA*, **105**, 11482–11489.

Dohmae, K., Koshimizu, U. & Nishimune, Y. (1993) In utero and mammary transfer of hantavirus antibody from dams to infant rats. *Laboratory Animal Science*, **43**, 557–561.

Dominik, S. (2005) Quantitative trait loci for internal nematode resistance in sheep: a review. *Genetics, Selection, Evolution*, **37**, S83-96.

Donnelly, P. (2008) Progress and challenges in genome-wide association studies in humans. *Nature*, **456**, 728–731.

Drobniak, S.M., Wiejaczka, D., Arct, A., Dubiec, A., Gustafsson, L. & Cichoń, M. (2010) Sex-specific heritability of cell-mediated immune response in the blue tit nestlings (*Cyanistes caeruleus*). *Journal of Evolutionary Biology*, **23**, 1286–1292.

Edfors-Lilja, I., Wattrang, E., Marklund, L., Moller, M., Andersson-Eklund, L., Andersson, L. & Fossum, C. (1998) Mapping quantitative trait loci for immune capacity in the pig. *Journal of Immunology*, **161**, 829–835.

Ellegren, H. (2014) Genome sequencing and population genomics in non-model organisms. *Trends in Ecology & Evolution*, **29**, 51–63.

Esser-von Bieren, J., Mosconi, I., Guiet, R., Piersgilli, A., Volpe, B., Chen, F., Gause, W.C., Seitz, A., Verbeek, J.S. & Harris, N.L. (2013) Antibodies trap tissue migrating helminth larvae and prevent tissue damage by driving IL-4Rα-independent alternative differentiation of macrophages. *PLoS Pathogens*, **9**, e1003771.

Evans, D.M., Frazer, I.H. & Martin, N.G. (1999) Genetic and environmental causes of variation in basal levels of blood cells. *Twin Research*, **2**, 250–257.

Falconer, D.S. & Mackay, T.F.C. (1996) *Introduction to Quantitative Genetics*, 4th Ed. Pearson Prentice Hall, Essex.

Ferreira, R.C., Pan-Hammarström, Q., Graham, R.R., Gateva, V., Fontán, G., Lee, A.T., Ortmann, W., Urcelay, E., Fernández-Arquero, M., Núñez, C., Jorgensen, G., Ludviksson, B.R., Koskinen, S., Haimila, K., Clark, H.F., Klareskog, L., Gregersen, P.K., Behrens, T.W. & Hammarström, L. (2010) Association of IFIH1 and other autoimmunity risk alleles with selective IgA deficiency. *Nature Genetics*, **42**, 777–780.

Feulner, P.G.D., Gratten, J., Kijas, J.W., Visscher, P.M., Pemberton, J.M. & Slate, J. (2013) Introgression and the fate of domesticated genes in a wild mammal population. *Molecular Ecology*, **22**, 4210–4221.

Fisher, R.A. (1930) *The Genetical Theory of Natural Selection*. The Clarendon Press, Oxford.

Flori, L., Gao, Y., Oswald, I.P., Lefevre, F., Bouffaud, M., Mercat, M.-J., Bidanel, J.-P. & Rogel-Gaillard, C. (2011) Deciphering the genetic control of innate and adaptive immune responses in pig: a combined genetic and genomic study. *BMC Proceedings*, **5**, S32.

Fox, C.W. & Mousseau, T.A. (1998) Maternal effects as adaptations for transgenerational phenotypic plasticity in insects. *Maternal effects as adaptations* (eds T.A. Mousseau & C.W. Fox), pp. 159–177. Oxford University Press, New York.

Fumagalli, M., Sironi, M., Pozzoli, U., Ferrer-Admettla, A., Pattini, L. & Nielsen, R. (2011) Signatures of environmental genetic adaptation pinpoint pathogens as the main selective pressure through human evolution. *PLoS Genetics*, **7**, e1002355.

Gangoso, L., Grande, J.M., Ducrest, A.-L., Figuerola, J., Bortolotti, G.R., Andrés, J.A. & Roulin, A. (2011) MC1R-dependent, melanin-based colour polymorphism is associated with cell-mediated response in the Eleonora's falcon. *Journal of Evolutionary Biology*, **24**, 2055–2063.

Gasparini, J., Bize, P., Piault, R., Wakamatsu, K., Blount, J.D., Ducrest, A.-L. & Roulin, A. (2009) Strength and cost of an induced immune response are associated with a heritable melanin-based colour trait in female tawny owls. *Journal of Animal Ecology*, **78**, 608–616.

Gasparini, J., McCoy, K.D., Haussy, C., Tveraa, T. & Boulinier, T. (2001) Induced maternal response to the Lyme disease spirochaete *Borrelia burgdorferi sensu lato* in a colonial seabird, the kittiwake *Rissa tridactyla*. *Proceedings of the Royal Society of London B: Biological Sciences*, **268**, 647–650.

Gauly, M. & Erhardt, G. (2001) Genetic resistance to gastrointestinal nematode parasites in Rhön sheep following natural infection. *Veterinary Parasitology*, **102**, 253–259.

Gause, W.C., Urban, J.F. & Stadecker, M.J. (2003) The immune response to parasitic helminths: insights from murine models. *Trends in Immunology*, **24**, 269–77.

Gilbert, R.P., Gaskins, C.T., Hillers, J.K., Parker, C.F. & McGuire, T.C. (1988) Genetic and environmental factors affecting immunoglobulin G1 concentrations in ewe colostrum and lamb serum. *Journal of Animal Science*, **66**, 855–863.

Gonzalez, G., Sorci, G., Møller, A.P., Ninni, P., Haussy, C. & De Lope, F. (1999) Immunocompetence and condition-dependent sexual advertisement in male house sparrows (*Passer domesticus*). *Journal of Animal Ecology*, **68**, 1225–1234.

Graham, A.L., Allen, J.E. & Read, A.F. (2005) Evolutionary causes and consequences of immunopathology. *Annual Review of Ecology, Evolution, and Systematics*, **36**, 373–397.

Graham, A.L., Hayward, A.D., Watt, K.A., Pilkington, J.G., Pemberton, J.M. & Nussey, D.H. (2010) Fitness correlates of heritable variation in antibody responsiveness in a wild mammal. *Science*, **330**, 662–665.

Graham, A.L., Nussey, D.H., Lloyd-Smith, J.O., Longbottom, D., Maley, M., Pemberton, J.M., Pilkington, J.G., Prager, K.C., Smith, L., Watt, K.A., Wilson, K., McNeilly, T.N. & Brülisauer, F. (2016) Exposure to viral and bacterial pathogens among Soay sheep (*Ovis aries*) of the St Kilda archipelago. *Epidemiology and Infection*, **144**, 1879–1888.

Graham, A.L., Shuker, D.M., Pollitt, L.C., Auld, S.K.J.R., Wilson, A.J. & Little, T.J. (2011) Fitness consequences of immune responses: strengthening the empirical framework for ecoimmunology. *Functional Ecology*, **25**, 5–17.

Grenfell, B.T., Wilson, K., Isham, V.S., Boyd, H.E.G. & Dietz, K. (1995) Modelling patterns of parasite aggregation in natural populations: trichostrongylid nematode–ruminant interactions as a case study. *Parasitology*, **111**, S135-151.

Grindstaff, J.L. (2008) Maternal antibodies reduce costs of an immune response during development. *The Journal of Experimental Biology*, **211**, 654–660.

Grindstaff, J.L., Brodie, E.D. & Ketterson, E.D. (2003) Immune function across generations: integrating mechanism and evolutionary process in maternal antibody transmission. *Proceedings of the Royal Society of London B: Biological Sciences*, **270**, 2309–2319.

Grindstaff, J.L., Hasselquist, D., Nilsson, J.-A., Sandell, M., Smith, H.G. & Stjernman, M. (2006) Transgenerational priming of immunity: maternal exposure to a bacterial antigen enhances offspring humoral immunity. *Proceedings of the Royal Society of London B: Biological Sciences*, **273**, 2551–2557.

Gulland, F.M. (1992) The role of nematode parasites in Soay sheep (*Ovis aries* L.) mortality

during a population crash. *Parasitology*, **105**, 493–503.

Gulland, F.M. & Fox, M. (1992) Epidemiology of nematode infections of Soay sheep (*Ovis aries* L.) on St Kilda. *Parasitology*, **105**, 481–492.

Gulliksen, S.M., Lie, K.I., Sølverød, L. & Østerås, O. (2008) Risk factors associated with colostrum quality in Norwegian dairy cows. *Journal of Dairy Science*, **91**, 704–712.

Guo, Z., González, J.F., Hernandez, J.N., McNeilly, T.N., Corripio-Miyar, Y., Frew, D., Morrison, T., Yu, P. & Li, R.W. (2016) Possible mechanisms of host resistance to Haemonchus contortus infection in sheep breeds native to the Canary Islands. *Scientific Reports*, **6**, 26200.

Gurish, M.F., Bryce, P.J., Tao, H., Kisselgof, A.B., Thornton, E.M., Miller, H.R., Friend, D.S. & Oettgen, H.C. (2004) IgE enhances parasite clearance and regulates mast cell responses in mice infected with *Trichinella spiralis*. *Journal of Immunology*, **172**, 1139–1145.

Gustafsson, E., Mattsson, A., Holmdahl, R. & Mattsson, R. (1994) Pregnancy in B-cell-deficient mice: postpartum transfer of immunoglobulins prevents neonatal runting and death. *Biology of Reproduction*, **51**, 1173–1180.

Gutiérrez-Gil, B., Pérez, J., Alvarez, L., Martínez-Valladares, M., de la Fuente, L.-F., Bayón, Y., Meana, A., San Primitivo, F., Rojo-Vázquez, F.-A. & Arranz, J.-J. (2009) Quantitative trait loci for resistance to trichostrongylid infection in Spanish Churra sheep. *Genetics, Selection, Evolution*, **41**, 46.

Gutiérrez-Gil, B., Pérez, J., de la Fuente, L.F., Meana, A., Martínez-Valladares, M., San Primitivo, F., Rojo-Vázquez, F.A. & Arranz, J.J. (2010) Genetic parameters for resistance to trichostrongylid infection in dairy sheep. *Animal*, **4**, 505–512.

Hargitai, R., Prechl, J. & Török, J. (2006) Maternal immunoglobulin concentration in Collared Flycatcher (*Ficedula albicollis*) eggs in relation to parental quality and laying order. *Functional Ecology*, **20**, 829–838.

Harris, N. & Gause, W. (2011) To B or not to B: B cells and the Th2-type immune response to helminths. *Trends in Immunology*, **32**, 80–88.

Harris, N.L., Spoerri, I., Schopfer, J.F., Nembrini, C., Merky, P., Massacand, J., Urban, J.F., Lamarre, A., Burki, K., Odermatt, B., Zinkernagel, R.M. & Macpherson, A.J. (2006) Mechanisms of neonatal mucosal antibody protection. *Journal of Immunology*, **177**, 6256–6262.

Hasselquist, D. & Nilsson, J.-A. (2009) Maternal transfer of antibodies in vertebrates: trans-generational effects on offspring immunity. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **364**, 51–60.

Hasselquist, D., Tobler, M. & Nilsson, J.-A. (2012) Maternal modulation of offspring immune function in vertebrates. *Ecoimmunology* (eds G.E. Demas & R.J. Nelson), pp. 165–224. Oxford University Press, New York.

Hayward, A.D., Garnier, R., Watt, K.A., Pilkington, J.G., Grenfell, B.T., Matthews, J.B., Pemberton, J.M., Nussey, D.H. & Graham, A.L. (2014) Heritable, heterogeneous, and costly resistance of sheep against nematodes and potential feedbacks to epidemiological dynamics. *The American Naturalist*, **184**, S58-76.

Hayward, A.D., Moorad, J., Regan, C.E., Berenos, C., Pilkington, J.G., Pemberton, J.M. & Nussey, D.H. (2015) Asynchrony of senescence among phenotypic traits in a wild

mammal population. *Experimental Gerontology*, **71**, 56–68.

Hayward, A.D., Wilson, A.J., Pilkington, J.G., Clutton-Brock, T.H., Pemberton, J.M. & Kruuk, L.E.B. (2011) Natural selection on a measure of parasite resistance varies across ages and environmental conditions in a wild mammal. *Journal of Evolutionary Biology*, **24**, 1664–1676.

Hayward, A.D., Wilson, A.J., Pilkington, J.G., Clutton-Brock, T.H., Pemberton, J.M. & Kruuk, L.E.B. (2013) Reproductive senescence in female Soay sheep: variation across traits and contributions of individual ageing and selective disappearance (ed C Fox). *Functional Ecology*, **27**, 184–195.

Hayward, A.D., Wilson, A.J., Pilkington, J.G., Pemberton, J.M. & Kruuk, L.E.B. (2009) Ageing in a variable habitat: environmental stress affects senescence in parasite resistance in St Kilda Soay sheep. *Proceedings of the Royal Society of London B: Biological Sciences*, **276**, 3477–3485.

Heeb, P., Werner, I., Kolliker, M. & Richner, H. (1998) Benefits of induced host responses against an ectoparasite. *Proceedings of the Royal Society of London B: Biological Sciences*, **265**, 51–56.

Henderson, N.G. & Stear, M.J. (2006) Eosinophil and IgA responses in sheep infected with *Teladorsagia circumcincta*. *Veterinary Immunology and Immunopathology*, **112**, 62–66.

Hernández-Castellano, L.E., Suárez-Trujillo, A., Martell-Jaizme, D., Cugno, G., Argüello, A. & Castro, N. (2015) The effect of colostrum period management on BW and immune system in lambs: from birth to weaning. *Animal*, **9**, 1672–1679.

Heyman, B. (2001) Functions of antibodies in the regulation of B cell responses in vivo. *Springer Seminars in Immunopathology*, **23**, 421–432.

Hindorff, L.A., Sethupathy, P., Junkins, H.A., Ramos, E.M., Mehta, J.P., Collins, F.S. & Manolio, T.A. (2009) Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. *Proceedings of the National Academy of Sciences of the USA*, **106**, 9362–9367.

Hine, B.C., Hunt, P.W., Beasley, A.M., Windon, R.G., Glover, S.A. & Colditz, I.G. (2010) Selective transport of IgE into ovine mammary secretions. *Research in Veterinary Science*, **89**, 184–190.

Hotez, P.J., Brindley, P.J., Bethony, J.M., King, C.H., Pearce, E.J. & Jacobson, J. (2008) Helminth infections: the great neglected tropical diseases. *The Journal of Clinical Investigation*, **118**, 1311–1321.

Hudson, P.J., Dobson, A.P. & Newborn, D. (1998) Prevention of population cycles by parasite removal. *Science*, **282**, 2256–2258.

Huisman, J. (2017) Pedigree reconstruction from SNP data: parentage assignment, sibship clustering and beyond. *Molecular Ecology Resources [Online early]*.

Huntley, J.F., Redmond, J., Welfare, W., Brennan, G., Jackson, F., Kooyman, F. & Vervelde, L. (2001) Studies on the immunoglobulin E responses to *Teladorsagia circumcincta* in sheep: purification of a major high molecular weight allergen. *Parasite Immunology*, **23**, 227–235.

Hurley, W.L. & Theil, P.K. (2011) Perspectives on immunoglobulins in colostrum and milk. *Nutrients*, **3**, 442–474.

Husband, A.J., Brandon, M.R. & Lascelles, A.K. (1972) Absorption and endogenous production of immunoglobulins in calves. *The Australian Journal of Experimental Biology and Medical Science*, **50**, 491–498.

Husby, A., Kawakami, T., Ronnegard, L., Smeds, L., Ellegren, H. & Qvarnstrom, A. (2015) Genome-wide association mapping in a wild avian population identifies a link between genetic and phenotypic variation in a life-history trait. *Proceedings of the Royal Society of London B: Biological Sciences*, **282**, 20150156.

Ilmonen, P., Taarna, T. & Hasselquist, D. (2000) Experimentally activated immune defence in female pied flycatchers results in reduced breeding success. *Proceedings of the Royal Society of London B: Biological Sciences*, **267**, 665–670.

Jackson, J.A., Hall, A.J., Friberg, I.M., Ralli, C., Lowe, A., Zawadzka, M., Turner, A.K., Stewart, A., Birtles, R.J., Paterson, S., Bradley, J.E. & Begon, M. (2014) An immunological marker of tolerance to infection in wild rodents. *PLoS Biology*, **12**, e1001901.

Jackson, F. & Miller, J. (2006) Alternative approaches to control—Quo vadit? *Veterinary Parasitology*, **139**, 371–384.

Jelonek, M.T., Maskrey, J.L., Steimer, K.S., Potts, B.J., Higgins, K.W. & Keller, M.A. (1996) Maternal monoclonal antibody to the V3 loop alters specificity of the response to a human immunodeficiency virus vaccine. *The Journal of Infectious Diseases*, **174**, 866–869.

Jepson, A., Banya, W., Sisay-Joof, F., Hassan-King, M., Nunes, C., Bennett, S. & Whittle, H. (1997) Quantification of the relative contribution of major histocompatibility complex (MHC) and non-MHC genes to human immune responses to foreign antigens. *Infection and Immunity*, **65**, 872–876.

Jiang, Y., Xie, M., Chen, W., Talbot, R., Maddox, J.F., Faraut, T., Wu, C., Muzny, D.M., Li, Y., Zhang, W., Stanton, J.-A., Brauning, R., Barris, W.C., Hourlier, T., Aken, B.L., Searle, S.M.J., Adelson, D.L., Bian, C., Cam, G.R., Chen, Y., Cheng, S., DeSilva, U., Dixen, K., Dong, Y., Fan, G., Franklin, I.R., Fu, S., Guan, R., Highland, M.A., Holder, M.E., Huang, G., Ingham, A.B., Jhangiani, S.N., Kalra, D., Kovar, C.L., Lee, S.L., Liu, W., Liu, X., Lu, C., Lv, T., Mathew, T., McWilliam, S., Menzies, M., Pan, S., Robelin, D., Servin, B., Townley, D., Wang, W., Wei, B., White, S.N., Yang, X., Ye, C., Yue, Y., Zeng, P., Zhou, Q., Hansen, J.B., Kristensen, K., Gibbs, R.A., Flicek, P., Warkup, C.C., Jones, H.E., Oddy, V.H., Nicholas, F.W., McEwan, J.C., Kijas, J., Wang, J., Worley, K.C., Archibald, A.L., Cockett, N., Xu, X., Wang, W. & Dalrymple, B.P. (2014) The sheep genome illuminates biology of the rumen and lipid metabolism. *Science*, **344**, 1168–1173.

Johnston, S.E., Bérénos, C., Slate, J. & Pemberton, J.M. (2016) Conserved genetic architecture underlying individual recombination rate variation in a wild population of Soay sheep (*Ovis aries*). *Genetics*, **203**, 583–598.

Johnston, S.E., Gratten, J., Bérénos, C., Pilkington, J.G., Clutton-Brock, T.H., Pemberton, J.M. & Slate, J. (2013) Life history trade-offs at a single locus maintain sexually selected genetic variation. *Nature*, **502**, 93–95.

Johnston, S.E., McEwan, J.C., Pickering, N.K., Kijas, J.W., Beraldi, D., Pilkington, J.G., Pemberton, J.M. & Slate, J. (2011) Genome-wide association mapping identifies the genetic basis of discrete and quantitative variation in sexual weaponry in a wild sheep population. *Molecular Ecology*, **20**, 2555–2566.

Johnston, S.E., Orell, P., Pritchard, V.L., Kent, M.P., Lien, S., Niemelä, E., Erkinaro, J. & Primmer, C.R. (2014) Genome-wide SNP analysis reveals a genetic basis for sea-age variation in a wild population of Atlantic salmon (*Salmo salar*). *Molecular Ecology*, **23**, 3452–3468.

Kacskovics, I. (2004) Fc receptors in livestock species. *Veterinary Immunology and Immunopathology*, **102**, 351–362.

Kallio, E.R., Poikonen, A., Vaheri, A., Vapalahti, O., Henttonen, H., Koskela, E. & Mappes, T. (2006) Maternal antibodies postpone hantavirus infection and enhance individual breeding success. *Proceedings of the Royal Society of London B: Biological Sciences*, **273**, 2771–2776.

Kardos, M., Husby, A., McFarlane, S.E., Qvarnström, A. & Ellegren, H. (2016) Whole-genome resequencing of extreme phenotypes in collared flycatchers highlights the difficulty of detecting quantitative trait loci in natural populations. *Molecular Ecology Resources*, **16**, 727–741.

Karell, P., Kontiainen, P., Pietiinen, H., Siitari, H. & Brommer, J.E. (2008) Maternal effects on offspring Igs and egg size in relation to natural and experimentally improved food supply. *Functional Ecology*, **22**, 682–690.

Kennedy, M.W. & Nager, R.G. (2006) The perils and prospects of using phytohaemagglutinin in evolutionary ecology. *Trends in Ecology & Evolution*, **21**, 653–655.

Kijas, J.W., Lenstra, J.A., Hayes, B., Boitard, S., Neto, L.R., Cristobal, M.S., Servin, B., McCulloch, R., Whan, V., Gietzen, K., Paiva, S., Barendse, W., Ciani, E., Raadsma, H., McEwan, J. & Dalrymple, B. (2012) Genome-wide analysis of the world's sheep breeds reveals high levels of historic mixture and strong recent selection. *PLoS Biology*, **10**, e1001258.

Kim, S.-Y., Fargallo, J.A., Vergara, P. & Martínez-Padilla, J. (2013) Multivariate heredity of melanin-based coloration, body mass and immunity. *Heredity*, **111**, 139–146.

Kirkpatrick, M. & Lande, R. (1989) The evolution of maternal characters. *Evolution*, **43**, 485–503.

Klasing, K.C. (1998) Nutritional modulation of resistance to infectious diseases. *Poultry Science*, **77**, 1119–1125.

Kraaijeveld, A.R. & Godfray, H.C.J. (1997) Trade-off between parasitoid resistance and larval competitive ability in *Drosophila melanogaster*. *Nature*, **389**, 278–280.

Kruse, V. (1970) Yield of colostrum and immunoglobulin in cattle at the first milking after parturition. *Animal Production*, **12**, 619–626.

Kruuk, L.E.B. (2004) Estimating genetic parameters in natural populations using the "animal model." *Philosophical Transactions of the Royal Society B: Biological Sciences*, **359**, 873–890.

Kruuk, L.E., Clutton-Brock, T.H., Slate, J., Pemberton, J.M., Brotherstone, S. & Guinness, F.E. (2000) Heritability of fitness in a wild mammal population. *Proceedings of the National Academy of Sciences of the USA*, **97**, 698–703.

Kruuk, L.E.B. & Hadfield, J.D. (2007) How to separate genetic and environmental causes of similarity between relatives. *Journal of Evolutionary Biology*, **20**, 1890–1903.

Kruuk, L.E.B., Livingston, J., Kahn, A. & Jennions, M.D. (2015) Sex-specific maternal effects in a viviparous fish. *Biology Letters*, **11**, 20150472.

Kruuk, L.E.B., Slate, J. & Wilson, A.J. (2008) New answers for old questions: the evolutionary quantitative genetics of wild animal populations. *Annual Review of Ecology, Evolution, and Systematics*, **39**, 525–548.

Laegreid, W.W., Heaton, M.P., Keen, J.E., Grosse, W.M., Chitko-McKown, C.G., Smith, T.P.L., Keele, J.W., Bennett, G.L. & Besser, T.E. (2002) Association of bovine neonatal Fc receptor α-chain gene (FCGRT) haplotypes with serum IgG concentration in newborn calves. *Mammalian Genome*, **13**, 704–710.

Lande, R. (1982) A quantitative genetic theory of life history evolution. *Ecology*, **63**, 607–615.

Langer, P. (2008) The phases of maternal investment in eutherian mammals. *Zoology*, **111**, 148–162.

Lazzaro, B.P. & Little, T.J. (2009) Immunity in a variable world. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **364**, 12–26.

Lemke, H., Coutinho, A. & Lange, H. (2004) Lamarckian inheritance by somatically acquired maternal IgG phenotypes. *Trends in Immunology*, **25**, 180–186.

Lemke, H. & Lange, H. (1999) Is there a maternally induced immunological imprinting phase à la Konrad Lorenz? *Scandinavian Journal of Immunology*, **50**, 348–354.

Lemke, H., Lange, H. & Berek, C. (1994) Maternal immunization modulates the primary immune response to 2-phenyl-oxazolone in BALB/c mice. *European Journal of Immunology*, **24**, 3025–3030.

Li, R.W., Hou, Y., Li, C. & Gasbarre, L.C. (2010) Localized complement activation in the development of protective immunity against *Ostertagia ostertagi* infections in cattle. *Veterinary Parasitology*, **174**, 247–256.

Li, J., Jørgensen, S.F., Maggadottir, S.M., Bakay, M., Warnatz, K., Glessner, J., Pandey, R., Salzer, U., Schmidt, R.E., Perez, E., Resnick, E., Goldacker, S., Buchta, M., Witte, T., Padyukov, L., Videm, V., Folseraas, T., Atschekzei, F., Elder, J.T., Nair, R.P., Winkelmann, J., Gieger, C., Nöthen, M.M., Büning, C., Brand, S., Sullivan, K.E., Orange, J.S., Fevang, B., Schreiber, S., Lieb, W., Aukrust, P., Chapel, H., Cunningham-Rundles, C., Franke, A., Karlsen, T.H., Grimbacher, B., Hakonarson, H., Hammarström, L. & Ellinghaus, E. (2015) Association of CLEC16A with human common variable immunodeficiency disorder and role in murine B cells. *Nature Communications*, **6**, 6804.

Lindström, K.M., Foufopoulos, J., Pärn, H. & Wikelski, M. (2004) Immunological investments reflect parasite abundance in island populations of Darwin's finches. *Proceedings of the Royal Society of London B: Biological Sciences*, **271**, 1513–1519.

Liston, A., Carr, E.J. & Linterman, M.A. (2016) Shaping variation in the human immune system. *Trends in Immunology*, **37**, 637–646.

Liu, Q., Kreider, T., Bowdridge, S., Liu, Z., Song, Y., Gaydo, A.G., Urban, J.F. & Gause, W.C. (2010) B cells have distinct roles in host protection against different nematode parasites. *The Journal of Immunology*, **184**, 5213–5223.

Lochmiller, R.L. & Deerenberg, C. (2000) Trade-offs in evolutionary immunology: just what is the cost of immunity? *Oikos*, **88**, 87–98.

Lu, X., Liu, J.F., Fu, W.X., Zhou, J.P., Luo, Y.R., Ding, X.D., Liu, Y. & Zhang, Q. (2013) Genome-wide association study for cytokines and immunoglobulin G in swine. *PLoS ONE*, **8**, 1–7.

Lundin, B.S., Dahlman-Höglund, A., Pettersson, I., Dahlgren, U.I.H., Hanson, L.Å. & Telemo, E. (1999) Antibodies given orally in the neonatal period can affect the immune response for two generations: evidence for active maternal influence on the newborn's immune system. *Scandinavian Journal of Immunology*, **50**, 651–656.

Lynch, M. & Walsh, B. (1998) *Genetics and Analysis of Quantitative Traits*. Sinauer, Sunderland, MA.

Maden, M., Altunok, V., Birdane, F.M., Aslan, V. & Nizamlioglu, M. (2003) Blood and colostrum/milk serum gamma-glutamyltransferase activity as a predictor of passive transfer status in lambs. *Journal of Veterinary Medicine Series B-Infectious Diseases and Veterinary Public Health*, **50**, 128–131.

Maestripieri, D. & Mateo, J.M. (2009) The role of maternal effects in mammalian evolution and adaptation. *Maternal Effects in Mammals* (eds D. Maestripieri & J.M. Mateo), pp. 1–10. University of Chicago Press, Chicago.

Maher, B. (2008) Personal genomes: the case of the missing heritability. *Nature*, **456**, 18–21.

Maizels, R.M. & Nussey, D.H. (2013) Into the wild: digging at immunology's evolutionary roots. *Nature Immunology*, **14**, 879–883.

Maizels, R.M. & Yazdanbakhsh, M. (2003) Immune regulation by helminth parasites: cellular and molecular mechanisms. *Nature Reviews Immunology*, **3**, 733–744.

Manolio, T.A., Collins, F.S., Cox, N.J., Goldstein, D.B., Hindorff, L.A., Hunter, D.J., McCarthy, M.I., Ramos, E.M., Cardon, L.R., Chakravarti, A., Cho, J.H., Guttmacher, A.E., Kong, A., Kruglyak, L., Mardis, E., Rotimi, C.N., Slatkin, M., Valle, D., Whittemore, A.S., Boehnke, M., Clark, A.G., Eichler, E.E., Gibson, G., Haines, J.L., Mackay, T.F.C., McCarroll, S.A. & Visscher, P.M. (2009) Finding the missing heritability of complex diseases. *Nature*, **461**, 747–753.

Martin, A., Dunnington, E.A., Gross, W.B., Briles, W.E., Briles, R.W. & Siegel, P.B. (1990) Production traits and alloantigen systems in lines of chickens selected for high or low antibody responses to sheep erythrocytes. *Poultry Science*, **69**, 871–878.

Martin, C., Saeftel, M., Vuong, P.N., Babayan, S., Fischer, K., Bain, O. & Hoerauf, A. (2001) B-cell deficiency suppresses vaccine-induced protection against murine filariasis but does not increase the recovery rate for primary infection. *Infection and Immunity*, **69**, 7067–7073.

Maruyama, H. & Nawa, Y. (2002) Immunology of nematode infection. *The Biology of Nematodes* (ed D.L. Lee), pp. 882–931. Taylor & Francis, London.

Marzal, A., Reviriego, M., de Lope, F. & Møller, A.P. (2007) Fitness costs of an immune response in the house martin (*Delichon urbica*). *Behavioral Ecology and Sociobiology*, **61**, 1573–1580.

Massimini, G., Peli, A., Boari, A. & Britti, D. (2006) Evaluation of assay procedures for prediction of passive transfer status in lambs. *American Journal of Veterinary Research*, **67**, 593–598.

Matson, K.D., Cohen, A.A., Klasing, K.C., Ricklefs, R.E. & Scheuerlein, A. (2006) No simple answers for ecological immunology: relationships among immune indices at the

individual level break down at the species level in waterfowl. *Proceedings of the Royal Society of London B: Biological Sciences*, **273**, 815–822.

May, R.M. & Anderson, R.M. (1978) Regulation and stability of host-parasite population interactions: II. Destabilizing processes. *Journal of Animal Ecology*, **47**, 249–267.

Mayer, B., Zolnai, A., Frenyó, L. V., Jancsik, V., Szentirmay, Z., Hammarström, L. & Kacskovics, I. (2002) Redistribution of the sheep neonatal Fc receptor in the mammary gland around the time of parturition in ewes and its localization in the small intestine of neonatal lambs. *Immunology*, **107**, 288–296.

McBean, D., Nath, M., Kenyon, F., Zile, K., Bartley, D.J. & Jackson, F. (2016) Faecal egg counts and immune markers in a line of Scottish Cashmere goats selected for resistance to gastrointestinal nematode parasite infection. *Veterinary Parasitology*, **229**, 1–8.

McCoy, K.D., Stoel, M., Stettler, R., Merky, P., Fink, K., Senn, B.M., Schaer, C., Massacand, J., Odermatt, B., Oettgen, H.C., Zinkernagel, R.M., Bos, N.A., Hengartner, H., Macpherson, A.J. & Harris, N.L. (2008) Polyclonal and specific antibodies mediate protective immunity against enteric helminth infection. *Cell Host & Microbe*, **4**, 362–373.

McMillen, I.C. & Robinson, J.S. (2005) Developmental origins of the metabolic syndrome: prediction, plasticity, and programming. *Physiological Reviews*, **85**, 571–633.

McNeilly, T.N., Devaney, E. & Matthews, J.B. (2009) *Teladorsagia circumcincta* in the sheep abomasum: defining the role of dendritic cells in T cell regulation and protective immunity. *Parasite Immunology*, **31**, 347–356.

McRae, K.M., Stear, M.J., Good, B. & Keane, O.M. (2015) The host immune response to gastrointestinal nematode infection in sheep. *Parasite Immunology*, **37**, 605–613.

Medzhitov, R., Schneider, D.S. & Soares, M.P. (2012) Disease tolerance as a defense strategy. *Science*, **335**, 936–941.

Merilä, J., Sheldon, B.C. & Kruuk, L.E. (2001) Explaining stasis: microevolutionary studies in natural populations. *Genetica*, **112**–**113**, 199–222.

Merino, S., Møller, A.P. & de Lope, F. (2000) Seasonal changes in cell-mediated immunocompetence and mass gain in nestling barn swallows: a parasite-mediated effect? *Oikos*, **90**, 327–332.

Michalek, S.M., Rahman, A.F. & McGhee, J.R. (1975) Rat immunoglobulins in serum and secretions: comparison of IgM, IgA and IgG in serum, colostrum, milk and saliva of protein malnourished and normal rats. *Proceedings of the Society for Experimental Biology and Medicine*, **148**, 1114–1118.

Morales, J., Sanz, J.J. & Moreno, J. (2006) Egg colour reflects the amount of yolk maternal antibodies and fledging success in a songbird. *Biology Letters*, **2**, 334–336.

Moreno, J., Lobato, E., Morales, J., Merino, S., Martínez-De La Puente, J. & Tomás, G. (2008) Pre-laying nutrition mediates maternal effects on offspring immune capacity and growth in the pied flycatcher. *Oecologia*, **156**, 727–735.

Morrison, E.S., Ardia, D.R. & Clotfelter, E.D. (2009) Cross-fostering reveals sources of variation in innate immunity and hematocrit in nestling tree swallows *Tachycineta bicolor*. *Journal of Avian Biology*, **40**, 573–578.

Moskvina, V. & Schmidt, K.M. (2008) On multiple-testing correction in genome-wide

association studies. *Genetic Epidemiology*, **32**, 567–573.

De Moura, E.G. & Passos, M.C.F. (2005) Neonatal programming of body weight regulation and energetic metabolism. *Bioscience Reports*, **25**, 251–269.

Mousseau, T.A. & Fox, C.W. (1998) The adaptive significance of maternal effects. *Trends in Ecology & Evolution*, **13**, 403–406.

Muggli, N.E., Hohenboken, W.D., Cundiff, L. V. & Kelley, K.W. (1984) Inheritance of maternal immunoglobulin G1 concentration by the bovine neonate. *Journal of Animal Science*, **59**, 39–48.

Muller, L.D. & Ellinger, D.K. (1981) Colostral immunoglobulin concentrations among breeds of dairy cattle. *Journal of Dairy Science*, **64**, 1727–1730.

Murphy, K.M. (2012) *Janeway's Immunobiology*, 8th Ed. Garland Science, Abingdon.

Murphy, L., Eckersall, P.D., Bishop, S.C., Pettit, J.J., Huntley, J.F., Burchmore, R. & Stear, M.J. (2010) Genetic variation among lambs in peripheral IgE activity against the larval stages of *Teladorsagia circumcincta*. *Parasitology*, **137**, 1249–1260.

Murray, C.J.L., Vos, T., Lozano, R., Naghavi, M., Flaxman, A.D., Michaud, C., Ezzati, M., Shibuya, K., Salomon, J.A., Abdalla, S., Aboyans, V., Abraham, J., Ackerman, I., Aggarwal, R., Ahn, S.Y., Ali, M.K., AlMazroa, M.A., Alvarado, M., Anderson, H.R., Anderson, L.M., Andrews, K.G., Atkinson, C., Baddour, L.M., Bahalim, A.N., Barker-Collo, S., Barrero, L.H., Bartels, D.H., Basáñez, M.-G., Baxter, A., Bell, M.L., Benjamin, E.J., Bennett, D., Bernabé, E., Bhalla, K., Bhandari, B., Bikbov, B., Abdulhak, A. Bin, Birbeck, G., Black, J.A., Blencowe, H., Blore, J.D., Blyth, F., Bolliger, I., Bonaventure, A., Boufous, S., Bourne, R., Boussinesq, M., Braithwaite, T., Brayne, C., Bridgett, L., Brooker, S., Brooks, P., Brugha, T.S., Bryan-Hancock, C., Bucello, C., Buchbinder, R., Buckle, G., Budke, C.M., Burch, M., Burney, P., Burstein, R., Calabria, B., Campbell, B., Canter, C.E., Carabin, H., Carapetis, J., Carmona, L., Cella, C., Charlson, F., Chen, H., Cheng, A.T.-A., Chou, D., Chugh, S.S., Coffeng, L.E., Colan, S.D., Colquhoun, S., Colson, K.E., Condon, J., Connor, M.D., Cooper, L.T., Corriere, M., Cortinovis, M., de Vaccaro, K.C., Couser, W., Cowie, B.C., Criqui, M.H., Cross, M., Dabhadkar, K.C., Dahiya, M., Dahodwala, N., Damsere-Derry, J., Danaei, G., Davis, A., Leo, D. De, Degenhardt, L., Dellavalle, R., Delossantos, A., Denenberg, J., Derrett, S., Des Jarlais, D.C., Dharmaratne, S.D., Dherani, M., Diaz-Torne, C., Dolk, H., Dorsey, E.R., Driscoll, T., Duber, H., Ebel, B., Edmond, K., Elbaz, A., Ali, S.E., Erskine, H., Erwin, P.J., Espindola, P., Ewoigbokhan, S.E., Farzadfar, F., Feigin, V., Felson, D.T., Ferrari, A., Ferri, C.P., Fèvre, E.M., Finucane, M.M., Flaxman, S., Flood, L., Foreman, K., Forouzanfar, M.H., Fowkes, F.G.R., Fransen, M., Freeman, M.K., Gabbe, B.J., Gabriel, S.E., Gakidou, E., Ganatra, H.A., Garcia, B., Gaspari, F., Gillum, R.F., Gmel, G., Gonzalez-Medina, D., Gosselin, R., Grainger, R., Grant, B., Groeger, J., Guillemin, F., Gunnell, D., Gupta, R., Haagsma, J., Hagan, H., Halasa, Y.A., Hall, W., Haring, D., Haro, J.M., Harrison, J.E., Havmoeller, R., Hay, R.J., Higashi, H., Hill, C., Hoen, B., Hoffman, H., Hotez, P.J., Hoy, D., Huang, J.J., Ibeanusi, S.E., Jacobsen, K.H., James, S.L., Jarvis, D., Jasrasaria, R., Jayaraman, S., Johns, N., Jonas, J.B., Karthikeyan, G., Kassebaum, N., Kawakami, N., Keren, A., Khoo, J.-P., King, C.H., Knowlton, L.M., Kobusingye, O., Koranteng, A., Krishnamurthi, R., Laden, F., Lalloo, R., Laslett, L.L., Lathlean, T., Leasher, J.L., Lee, Y.Y., Leigh, J., Levinson, D., Lim, S.S., Limb, E., Lin, J.K., Lipnick, M., Lipshultz, S.E., Liu, W., Loane, M., Ohno, S.L., Lyons, R., Mabweijano, J., MacIntyre, M.F., Malekzadeh, R., Mallinger, L., Manivannan, S., Marcenes, W., March, L., Margolis, D.J., Marks, G.B., Marks, R., Matsumori, A., Matzopoulos, R., Mayosi, B.M.,

McAnulty, J.H., McDermott, M.M., McGill, N., McGrath, J., Medina-Mora, M.E., Meltzer, M., Memish, Z.A., Mensah, G.A., Merriman, T.R., Meyer, A.-C., Miglioli, V., Miller, M., Miller, T.R., Mitchell, P.B., Mock, C., Mocumbi, A.O., Moffitt, T.E., Mokdad, A.A., Monasta, L., Montico, M., Moradi-Lakeh, M., Moran, A., Morawska, L., Mori, R., Murdoch, M.E., Mwaniki, M.K., Naidoo, K., Nair, M.N., Naldi, L., Narayan, K.M.V., Nelson, P.K., Nelson, R.G., Nevitt, M.C., Newton, C.R., Nolte, S., Norman, P., Norman, R., O'Donnell, M., O'Hanlon, S., Olives, C., Omer, S.B., Ortblad, K., Osborne, R., Ozgediz, D., Page, A., Pahari, B., Pandian, J.D., Rivero, A.P., Patten, S.B., Pearce, N., Padilla, R.P., Perez-Ruiz, F., Perico, N., Pesudovs, K., Phillips, D., Phillips, M.R., Pierce, K., Pion, S., Polanczyk, G. V, Polinder, S., Pope, C.A., Popova, S., Porrini, E., Pourmalek, F., Prince, M., Pullan, R.L., Ramaiah, K.D., Ranganathan, D., Razavi, H., Regan, M., Rehm, J.T., Rein, D.B., Remuzzi, G., Richardson, K., Rivara, F.P., Roberts, T., Robinson, C., De Leòn, F.R., Ronfani, L., Room, R., Rosenfeld, L.C., Rushton, L., Sacco, R.L., Saha, S., Sampson, U., Sanchez-Riera, L., Sanman, E., Schwebel, D.C., Scott, J.G., Segui-Gomez, M., Shahraz, S., Shepard, D.S., Shin, H., Shivakoti, R., Silberberg, D., Singh, D., Singh, G.M., Singh, J.A., Singleton, J., Sleet, D.A., Sliwa, K., Smith, E., Smith, J.L., Stapelberg, N.J., Steer, A., Steiner, T., Stolk, W.A., Stovner, L.J., Sudfeld, C., Syed, S., Tamburlini, G., Tavakkoli, M., Taylor, H.R., Taylor, J.A., Taylor, W.J., Thomas, B., Thomson, W.M., Thurston, G.D., Tleyjeh, I.M., Tonelli, M., Towbin, J.A., Truelsen, T., Tsilimbaris, M.K., Ubeda, C., Undurraga, E.A., van der Werf, M.J., van Os, J., Vavilala, M.S., Venketasubramanian, N., Wang, M., Wang, W., Watt, K., Weatherall, D.J., Weinstock, M.A., Weintraub, R., Weisskopf, M.G., Weissman, M.M., White, R.A., Whiteford, H., Wiebe, N., Wiersma, S.T., Wilkinson, J.D., Williams, H.C., Williams, S.R., Witt, E., Wolfe, F., Woolf, A.D., Wulf, S., Yeh, P.-H., Zaidi, A.K., Zheng, Z.-J., Zonies, D. & Lopez, A.D. (2012) Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. *The Lancet*, **380**, 2197–2223.

Negrão-Corrêa, D. (2001) Importance of immunoglobulin E (IgE) in the protective mechanism against gastrointestinal nematode infection: looking at the intestinal mucosae. *Revista do Instituto de Medicina Tropical de São Paulo*, **43**, 291–299.

Nieuwhof, G.J. & Bishop, S.C. (2005) Costs of the major endemic diseases of sheep in Great Britain and the potential benefits of reduction in disease impact. *Animal Science*, **81**, 23–29.

Nohynek, H., Gustafsson, L., Capeding, M.R., Käyhty, H., Olander, R.M., Pascualk, L. & Ruutu, P. (1999) Effect of transplacentally acquired tetanus antibodies on the antibody responses to *Haemophilus influenzae* type b-tetanus toxoid conjugate and tetanus toxoid vaccines in Filipino infants. *The Pediatric Infectious Disease Journal*, **18**, 25–30.

van Noordwijk, A.J. & de Jong, G. (1986) Acquisition and allocation of resources: their influence on variation in life history tactics. *The American Naturalist*, **128**, 137–142.

Norman, L.M., Hohenboken, W.D. & Kelley, K.W. (1981) Genetic differences in concentration of immunoglobulins G1 and M in serum and colostrum of cows and in serum of neonatal calves. *Journal of Animal Science*, **53**, 1465–1472.

Nunn, C.L., Gittleman, J.L. & Antonovics, J. (2000) Promiscuity and the primate immune system. *Science*, **290**, 1168–1170.

Nussey, D.H., Watt, K.A., Clark, A., Pilkington, J.G., Pemberton, J.M., Graham, A.L. & McNeilly, T.N. (2014) Multivariate immune defences and fitness in the wild: complex

but ecologically important associations among plasma antibodies, health and survival. *Proceedings of the Royal Society of London B: Biological Sciences*, **281**, 20132931.

Nussey, D.H., Watt, K., Pilkington, J.G., Zamoyska, R. & Mcneilly, T.N. (2012) Age-related variation in immunity in a wild mammal population. *Aging Cell*, **11**, 178–180.

Okamoto, Y., Tsutsumi, H., Kumar, N.S. & Ogra, P.L. (1989) Effect of breast feeding on the development of anti-idiotype antibody response to F glycoprotein of respiratory syncytial virus in infant mice after post-partum maternal immunization. *The Journal of Immunology*, **142**, 2507–2512.

Orrù, V., Steri, M., Sole, G., Sidore, C., Virdis, F., Dei, M., Lai, S., Zoledziewska, M., Busonero, F., Mulas, A., Floris, M., Mentzen, W.I., Urru, S.A.M., Olla, S., Marongiu, M., Piras, M.G., Lobina, M., Maschio, A., Pitzalis, M., Urru, M.F., Marcelli, M., Cusano, R., Deidda, F., Serra, V., Oppo, M., Pilu, R., Reinier, F., Berutti, R., Pireddu, L., Zara, I., Porcu, E., Kwong, A., Brennan, C., Tarrier, B., Lyons, R., Kang, H.M., Uzzau, S., Atzeni, R., Valentini, M., Firinu, D., Leoni, L., Rotta, G., Naitza, S., Angius, A., Congia, M., Whalen, M.B., Jones, C.M., Schlessinger, D., Abecasis, G.R., Fiorillo, E., Sanna, S. & Cucca, F. (2013) Genetic variants regulating immune cell levels in health and disease. *Cell*, **155**, 242–256.

Owen, J.P. & Clayton, D.H. (2007) Where are the parasites in the PHA response? *Trends in Ecology & Evolution*, **22**, 228–229.

Palacios, M.G., Winkler, D.W., Klasing, K.C., Hasselquist, D. & Vleck, C.M. (2011) Consequences of immune system aging in nature: a study of immunosenescence costs in free-living Tree Swallows. *Ecology*, **92**, 952–966.

Paoletti, L.C., Pinel, J., Kennedy, R.C. & Kasper, D.L. (2000) Maternal antibody transfer in baboons and mice vaccinated with a group B streptococcal polysaccharide conjugate. *The Journal of Infectious Diseases*, **181**, 653–658.

Parejo, D. & Silva, N. (2009) Immunity and fitness in a wild population of Eurasian kestrels *Falco tinnunculus*. *Naturwissenschaften*, **96**, 1193–1202.

Pastoret, P.-P., Griebel, P., Bazin, H. & Govaerts, A. (1998) *Handbook of Vertebrate Immunology*. Academic Press Limited, London.

Paterson, S., Wilson, K. & Pemberton, J.M. (1998) Major histocompatibility complex variation associated with juvenile survival and parasite resistance in a large unmanaged ungulate population. *Proceedings of the National Academy of Sciences of the USA*, **95**, 3714–3719.

Pedersen, A.B. & Babayan, S.A. (2011) Wild immunology. *Molecular Ecology*, **20**, 872–880.

Pedersen, A.B. & Greives, T.J. (2008) The interaction of parasites and resources cause crashes in a wild mouse population. *Journal of Animal Ecology*, **77**, 370–377.

Pemberton, J.M. (2008) Wild pedigrees: the way forward. *Proceedings of the Royal Society of London B: Biological Sciences*, **275**, 613–621.

Pfeffer, A., Shaw, R.J., Green, R.S. & Phegan, M.D. (2005) The transfer of maternal IgE and other immunoglobulins specific for *Trichostrongylus colubriformis* larval excretory/secretory product to the neonatal lamb. *Veterinary Immunology and Immunopathology*, **108**, 315–323.

Pihlaja, M., Siitari, H. & Alatalo, R.V. (2006) Maternal antibodies in a wild altricial bird:

effects on offspring immunity, growth and survival. *Journal of Animal Ecology*, **75**, 1154–1164.

Pitala, N., Gustafsson, L., Sendecka, J. & Brommer, J.E. (2007) Nestling immune response to phytohaemagglutinin is not heritable in collared flycatchers. *Biology Letters*, **3**, 418–421.

Postma, E. (2014) Four decades of estimating heritabilities in wild vertebrate populations: improved methods, more data, better estimates? *Quantitative Genetics in the Wild* (eds A. Charmantier, D. Garant & L.E.B. Kruuk), pp. 16–33. Oxford University Press, Oxford.

Potti, J. & Merino, S. (1994) Heritability estimates and maternal effects on tarsus length in pied flycatchers, *Ficedula hypoleuca*. *Oecologia*, **100**, 331–338.

Poulin, R. (2014) Parasite biodiversity revisited: frontiers and constraints. *International Journal for Parasitology*, **44**, 581–589.

Poulin, R. & Morand, S. (2004) *Parasite Biodiversity*. Smithsonian Institution Press, Washington.

Prada Jiménez de Cisneros, J., Stear, M.J., Mair, C., Singleton, D., Stefan, T., Stear, A., Marion, G. & Matthews, L. (2014) An explicit immunogenetic model of gastrointestinal nematode infection in sheep. *Journal of the Royal Society Interface*, **11**, 20140416.

Preston, B.T., Stevenson, I.R., Lincoln, G.A., Monfort, S.L., Pilkington, J.G. & Wilson, K. (2012) Testes size, testosterone production and reproductive behaviour in a natural mammalian mating system. *Journal of Animal Ecology*, **81**, 296–305.

Preston, B.T., Stevenson, I.R., Pemberton, J.M., Coltman, D.W. & Wilson, K. (2003) Overt and covert competition in a promiscuous mammal: the importance of weaponry and testes size to male reproductive success. *Proceedings of the Royal Society of London B: Biological Sciences*, **270**, 633–640.

Prugnolle, F., Manica, A., Charpentier, M., Guégan, J.F., Guernier, V. & Balloux, F. (2005) Pathogen-driven selection and worldwide HLA class I diversity. *Current Biology*, **15**, 1022–1027.

Pullan, R.L., Kabatereine, N.B., Quinnell, R.J. & Brooker, S. (2010) Spatial and genetic epidemiology of hookworm in a rural community in Uganda. *PLoS Neglected Tropical Diseases*, **4**, e713.

Quinnell, R.J. (2003) Genetics of susceptibility to human helminth infection. *International Journal for Parasitology*, **33**, 1219–1231.

Råberg, L., Graham, A.L. & Read, A.F. (2009) Decomposing health: tolerance and resistance to parasites in animals. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **364**, 37–49.

Råberg, L., Nilsson, J.-A., Ilmonen, P., Stjernman, M. & Hasselquist, D. (2000) The cost of an immune response: vaccination reduces parental effort. *Ecology Letters*, **3**, 382–386.

Råberg, L. & Stjernman, M. (2003) Natural selection on immune responsiveness in blue tits *Parus caeruleus*. *Evolution*, **57**, 1670–1678.

Råberg, L., Stjernman, M. & Hasselquist, D. (2003) Immune responsiveness in adult blue tits: heritability and effects of nutritional status during ontogeny. *Oecologia*, **136**, 360–

364.

Raboisson, D., Trillat, P. & Cahuzac, C. (2016) Failure of passive immune transfer in calves: a meta-analysis on the consequences and assessment of the economic impact. *PLoS ONE*, **11**, e0150452.

R Core Team. (2016) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Rajan, B., Ramalingam, T. & Rajan, T. V. (2005) Critical role for IgM in host protection in experimental filarial infection. *Journal of Immunology*, **175**, 1827–1833.

Räsänen, K. & Kruuk, L.E.B. (2007) Maternal effects and evolution at ecological time-scales. *Functional Ecology*, **21**, 408–421.

Regan, C.E., Pilkington, J.G., Bérénos, C., Pemberton, J.M., Smiseth, P.T. & Wilson, A.J. (2017) Accounting for female space sharing in St. Kilda Soay sheep (*Ovis aries*) results in little change in heritability estimates. *Journal of Evolutionary Biology*, **30**, 96–111.

Regan, C.E., Pilkington, J.G., Pemberton, J.M. & Crawley, M.J. (2016) Sex differences in relationships between habitat use and reproductive performance in Soay sheep (Ovis aries). *Ecology Letters*, **19**, 171–179.

Reinhold, K. (2002) Maternal effects and the evolution of behavioral and morphological characters: a literature review indicates the importance of extended maternal care. *Journal of Heredity*, **93**, 400–405.

Reznick, D., Nunney, L. & Tessier, A. (2000) Big houses, big cars, superfleas and the costs of reproduction. *Trends in Ecology & Evolution*, **15**, 421–425.

El Ridi, R., Ozaki, T. & Kamiya, H. (1998) *Schistosoma mansoni* infection in IgE-producing and IgE-deficient mice. *The Journal of Parasitology*, **84**, 171–174.

Riggio, V., Matika, O., Pong-Wong, R., Stear, M.J. & Bishop, S.C. (2013) Genome-wide association and regional heritability mapping to identify loci underlying variation in nematode resistance and body weight in Scottish Blackface lambs. *Heredity*, **110**, 420–429.

Rincheval-Arnold, A., Belair, L., Cencic, A. & Djiane, J. (2002a) Up-regulation of polymeric immunoglobulin receptor mRNA in mammary epithelial cells by IFN-γ. *Molecular and Cellular Endocrinology*, **194**, 95–105.

Rincheval-Arnold, A., Belair, L. & Djiane, J. (2002b) Developmental expression of pIgR gene in sheep mammary gland and hormonal regulation. *The Journal of Dairy Research*, **69**, 13–26.

Robertson, A., Hiraiwa-Hasegawa, M., Albon, S.D. & Clutton-Brock, T.H. (1992) Early growth and suckling behavior of Soay sheep in a fluctuating population. *Journal of Zoology*, **227**, 661–671.

Robinson, M.R., Pilkington, J.G., Clutton-Brock, T.H., Pemberton, J.M. & Kruuk, L.E.B. (2006) Live fast, die young: trade-offs between fitness components and sexually antagonistic selection on weaponry in Soay sheep. *Evolution*, **60**, 2168–2181.

Robinson, M.R., Pilkington, J.G., Clutton-Brock, T.H., Pemberton, J.M. & Kruuk, L.E.B. (2008) Environmental heterogeneity generates fluctuating selection on a secondary sexual trait. *Current Biology*, **18**, 751–757.

Robison, J.D., Stott, G.H. & DeNise, S.K. (1988) Effects of passive immunity on growth and survival in the dairy heifer. *Journal of Dairy Science*, **71**, 1283–1287.

Roederer, M., Quaye, L., Mangino, M., Beddall, M.H., Mahnke, Y., Chattopadhyay, P., Tosi, I., Napolitano, L., Terranova Barberio, M., Menni, C., Villanova, F., Di Meglio, P., Spector, T.D. & Nestle, F.O. (2015) The genetic architecture of the human immune system: a bioresource for autoimmunity and disease pathogenesis. *Cell*, **161**, 387–403.

Rohrer, G.A., Rempel, L.A., Miles, J.R., Keele, J.W., Wiedmann, R.T. & Vallet, J.L. (2014) Identifying genetic loci controlling neonatal passive transfer of immunity using a hybrid genotyping strategy. *Animal Genetics*, **45**, 340–349.

Rönnegård, L., McFarlane, S.E., Husby, A., Kawakami, T., Ellegren, H. & Qvarnström, A. (2016) Increasing the power of genome wide association studies in natural populations using repeated measures - evaluation and implementation. *Methods in Ecology and Evolution*, **7**, 792–799.

Saino, N., Bolzern, A.M. & Møller, A.P. (1997) Immunocompetence, ornamentation, and viability of male barn swallows (*Hirundo rustica*). *Proceedings of the National Academy of Sciences of the USA*, **94**, 549–552.

Sakaluk, S.K., Wilson, A.J., Bowers, E.K., Johnson, L.S., Masters, B.S., Johnson, B.G., Vogel, L.A., Forsman, A.M. & Thompson, C.F. (2014) Genetic and environmental variation in condition, cutaneous immunity, and haematocrit in house wrens. *BMC Evolutionary Biology*, **14**, 242.

Sangild, P.T. (2003) Uptake of colostral immunoglobulins by the compromised newborn farm animal. *Acta Veterinaria Scandinavica. Supplementum*, **98**, 105–122.

Santure, A.W., Poissant, J., De Cauwer, I., Van Oers, K., Robinson, M.R., Quinn, J.L., Groenen, M.A.M., Visser, M.E., Sheldon, B.C. & Slate, J. (2015) Replicated analysis of the genetic architecture of quantitative traits in two wild great tit populations. *Molecular Ecology*, **24**, 6148–6162.

Scharsack, J.P., Kalbe, M., Harrod, C. & Rauch, G. (2007) Habitat-specific adaptation of immune responses of stickleback (*Gasterosteus aculeatus*) lake and river ecotypes. *Proceedings of the Royal Society of London B: Biological Sciences*, **274**, 1523–1532.

Schmid-Hempel, P. (2003) Variation in immune defence as a question of evolutionary ecology. *Proceedings of the Royal Society of London B: Biological Sciences*, **270**, 357–366.

Schmid-Hempel, P. (2011) *Evolutionary Parasitology: The Integrated Study of Infections, Immunology, Ecology, and Genetics*. Oxford University Press, New York.

Seppälä, O. (2015) Natural selection on quantitative immune defence traits: a comparison between theory and data. *Journal of Evolutionary Biology*, **28**, 1–9.

Seppälä, O. & Jokela, J. (2010) Maintenance of genetic variation in immune defense of a freshwater snail: role of environmental heterogeneity. *Evolution*, **64**, 2397–2407.

Shaw, D.J. & Dobson, A.P. (1995) Patterns of macroparasite abundance and aggregation in wildlife populations: a quantitative review. *Parasitology*, **111**, S111-127.

Sheldon, B.C. & Verhulst, S. (1996) Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends in Ecology & Evolution*, **11**, 317–321.

Shin, J.-H., Blay, S., McNeney, B. & Graham, J. (2006) LDheatmap: an R function for

graphical display of pairwise linkage disequilibria between single nucleotide polymorphisms. *Journal of Statistical Software*, **16**, Code Snippet 3.

Siegel, P.B., Gross, W.B. & Cherry, J.A. (2009) Correlated responses of chickens to selection for production of antibodies to sheep erythrocytes. *Animal Blood Groups and Biochemical Genetics*, **13**, 291–297.

Siegrist, C.A. (2003) Mechanisms by which maternal antibodies influence infant vaccine responses: review of hypotheses and definition of main determinants. *Vaccine*, **21**, 3406–3412.

Silva, C.N.S., McFarlane, S.E., Hagen, I.J., Rönnegård, L., Billing, A.M., Kvalnes, T., Kemppainen, P., Rønning, B., Ringsby, T.H., Sæther, B.-E., Qvarnström, A., Ellegren, H., Jensen, H. & Husby, A. (2017) Insights into the genetic architecture of morphological traits in two passerine bird species. *Heredity*, **119**, 197–205.

Simon, A.K., Hollander, G.A. & McMichael, A. (2015) Evolution of the immune system in humans from infancy to old age. *Proceedings of the Royal Society of London B: Biological Sciences*, **282**, 20143085.

Siva-Jothy, M.T. & Thompson, J.J.W. (2002) Short-term nutrient deprivation affects immune function. *Physiological Entomology*, **27**, 206–212.

Slate, J. (2005) Quantitative trait locus mapping in natural populations: progress, caveats and future directions. *Molecular Ecology*, **14**, 363–379.

Slate, J. (2013) From Beavis to beak color: a simulation study to examine how much QTL mapping can reveal about the genetic architecture of quantitative traits. *Evolution*, **67**, 1251–1262.

Slate, J., Santure, A.W., Feulner, P.G.D., Brown, E.A., Ball, A.D., Johnston, S.E. & Gratten, J. (2010) Genome mapping in intensively studied wild vertebrate populations. *Trends in Genetics*, **26**, 275–284.

Staszewski, V., Gasparini, J., McCoy, K.D., Tveraa, T. & Boulinier, T. (2007) Evidence of an interannual effect of maternal immunization on the immune response of juveniles in a long-lived colonial bird. *Journal of Animal Ecology*, **76**, 1215–1223.

Stear, M.J., Bairden, K., Innocent, G.T., Mitchell, S., Strain, S. & Bishop, S.C. (2004) The relationship between IgA activity against 4th-stage larvae and density-dependent effects on the number of 4th-stage larvae of *Teladorsagia circumcincta* in naturally infected sheep. *Parasitology*, **129**, 363–369.

Stear, M.J. & Bishop, S.C. (1999) The curvilinear relationship between worm length and fecundity of *Teladorsagia circumcincta*. *International Journal for Parasitology*, **29**, 777–780.

Stear, M.J., Bishop, S.C., Doligalska, M., Duncan, J.L., Holmes, P.H., Irvine, J., McCririe, L., McKellar, Q.A., Sinski, E. & Murray, M. (1995) Regulation of egg production, worm burden, worm length and worm fecundity by host responses in sheep infected with *Ostertagia circumcincta*. *Parasite Immunology*, **17**, 643–652.

Stear, M.J., Boag, B., Cattadori, I. & Murphy, L. (2009) Genetic variation in resistance to mixed, predominantly *Teladorsagia circumcincta* nematode infections of sheep: from heritabilities to gene identification. *Parasite Immunology*, **31**, 274–282.

Stear, M., Park, M. & Bishop, S. (1996) The key components of resistance to *Ostertagia circumcincta* in lambs. *Parasitology Today*, **12**, 438–441.

Stern, C.M.M. (1976) The materno-foetal transfer of carrier protein sensitivity in the mouse. *Immunology*, **30**, 443–448.

Strain, S.A.J., Bishop, S.C., Henderson, N.G., Kerr, A., McKellar, Q.A., Mitchell, S. & Stear, M.J. (2002) The genetic control of IgA activity against *Teladorsagia circumcincta* and its association with parasite resistance in naturally infected sheep. *Parasitology*, **124**, 545–552.

Svensson, E., Sinervo, B. & Comendant, T. (2001) Density-dependent competition and selection on immune function in genetic lizard morphs. *Proceedings of the National Academy of Sciences of the USA*, **98**, 12561–12565.

Tarka, M., Åkesson, M., Beraldi, D., Hernández-Sánchez, J., Hasselquist, D., Bensch, S. & Hansson, B. (2010) A strong quantitative trait locus for wing length on chromosome 2 in a wild population of great reed warblers. *Proceedings of the Royal Society of London B: Biological Sciences*, **277**, 2361–2369.

Tavecchia, G., Coulson, T., Morgan, B.J.T., Pemberton, J.M., Pilkington, J.C., Gulland, F.M.D. & Clutton-Brock, T.H. (2005) Predictors of reproductive cost in female Soay sheep. *Journal of Animal Ecology*, **74**, 201–213.

Thatcher, E.F. & Gershwin, L.J. (1989) Colostral transfer of bovine immunoglobulin E and dynamics of serum IgE in calves. *Veterinary Immunology and Immunopathology*, **20**, 325–334.

Thompson-Crispi, K.A., Sargolzaei, M., Ventura, R., Abo-Ismail, M., Miglior, F., Schenkel, F. & Mallard, B.A. (2014) A genome-wide association study of immune response traits in Canadian Holstein cattle. *BMC Genomics*, **15**, 559.

Thompson-Crispi, K.A., Sewalem, A., Miglior, F. & Mallard, B.A. (2012) Genetic parameters of adaptive immune response traits in Canadian Holsteins. *Journal of Dairy Science*, **95**, 401–409.

Tizard, I.R. (2012) *Veterinary Immunology*, 9th Ed. Elsevier, St. Louis, Missouri.

Troisi, C.L., Hollinger, F.B., Krause, D.S. & Pickering, L.K. (1997) Immunization of seronegative infants with hepatitis A vaccine (HAVRIX; SKB): a comparative study of two dosing schedules. *Vaccine*, **15**, 1613–1617.

Tsang, J.S., Schwartzberg, P.L., Kotliarov, Y., Biancotto, A., Xie, Z., Germain, R.N., Wang, E., Olnes, M.J., Narayanan, M., Golding, H., Moir, S., Dickler, H.B., Perl, S. & Cheung, F. (2014) Global analyses of human immune variation reveal baseline predictors of postvaccination responses. *Cell*, **157**, 499–513.

Tschirren, B., Siitari, H., Saladin, V. & Richner, H. (2009) Transgenerational immunity in a bird-ectoparasite system: do maternally transferred antibodies affect parasite fecundity or the offspring's susceptibility to fleas? *Ibis*, **151**, 160–170.

Turner, A.K., Begon, M., Jackson, J.A., Bradley, J.E. & Paterson, S. (2011) Genetic diversity in cytokines associated with immune variation and resistance to multiple pathogens in a natural rodent population. *PLoS Genetics*, **7**, e1002343.

Ubosi, C.O., Gross, W.B. & Siegel, P.B. (1985) Divergent selection of chickens for antibody production to sheep erythrocytes: age effect in parental lines and their crosses. *Avian Diseases*, **29**, 150–158.

Uller, T., Isaksson, C. & Olsson, M. (2006) Immune challenge reduces reproductive output and growth in a lizard. *Functional Ecology*, **20**, 873–879.

Viney, M.E., Riley, E.M. & Buchanan, K.L. (2005) Optimal immune responses: immunocompetence revisited. *Trends in Ecology & Evolution*, **20**, 665–669.

Walsh, B. & Blows, M.W. (2009) Abundant genetic variation + strong selection = multivariate genetic constraints: a geometric view of adaptation. *Annual Review of Ecology, Evolution, and Systematics*, **40**, 41–59.

Watanabe, N., Katakura, K., Kobayashi, A., Okumura, K. & Ovary, Z. (1988) Protective immunity and eosinophilia in IgE-deficient SJA/9 mice infected with *Nippostrongylus brasiliensis* and *Trichinella spiralis*. *Proceedings of the National Academy of Sciences of the USA*, **85**, 4460–4462.

Watson, R.L., McNeilly, T.N., Watt, K.A., Pemberton, J.M., Pilkington, J.G., Waterfall, M., Hopper, P.R.T., Cooney, D., Zamoyska, R. & Nussey, D.H. (2016) Cellular and humoral immunity in a wild mammal: variation with age & sex and association with overwinter survival. *Ecology & Evolution*, **6**, 8695–8705.

Weaver, D.M., Tyler, J.W., VanMetre, D.C., Hostetler, D.E. & Barrington, G.M. (2000) Passive transfer of colostral immunoglobulins in calves. *Journal of Veterinary Internal Medicine*, **14**, 569–577.

Wenzel, M.A., James, M.C., Douglas, A. & Piertney, S.B. (2015) Genome-wide association and genome partitioning reveal novel genomic regions underlying variation in gastrointestinal nematode burden in a wild bird. *Molecular Ecology*, **24**, 4175–4192.

WHO. (2017) Soil-transmitted helminth infections | Fact sheet. URL http://www.who.int/mediacentre/factsheets/fs366/en/ [accessed 27 August 2017]

Wilfert, L. & Schmid-Hempel, P. (2008) The genetic architecture of susceptibility to parasites. *BMC Evolutionary Biology*, **8**, 187.

Williams, A.R., Palmer, D.G., Williams, I.H., Vercoe, P.E., Emery, D.L. & Karlsson, L.J.E. (2010) Relationships between immune indicators of parasitic gastroenteritis, nematode burdens and faecal dry matter in sheep. *Animal Production Science*, **50**, 219–227.

Wilson, A.J. (2008) Why h2 does not always equal VA/VP? *Journal of Evolutionary Biology*, **21**, 647–650.

Wilson, K., Bjørnstad, O.N., Dobson, A.P., Merler, S., Poglayen, G., Randolph, S.E., Read, A.F. & Skorping, A. (2002) Heterogeneities in macroparasite infections: patterns and processes. *The Ecology of Wildlife Diseases* (eds P.J. Hudson, A. Rizzoli, B.T. Grenfell, H. Heesterbeek & A.P. Dobson), pp. 6–44. Oxford University Press, Oxford.

Wilson, A.J., Coltman, D.W., Pemberton, J.M., Overall, A.D.J., Byrne, K.A. & Kruuk, L.E.B. (2005) Maternal genetic effects set the potential for evolution in a free-living vertebrate population. *Journal of Evolutionary Biology*, **18**, 405–414.

Wilson, K., Grenfell, B.T., Pilkington, J.G., Boyd, H.E.G. & Gulland, F.M.. (2004) Parasites and their impact. *Soay Sheep: Dynamics and Selection in an Island Population* (eds T.H. Clutton-Brock & J.M. Pemberton), pp. 17–51. Cambridge University Press, Cambridge, UK.

Wilson, A.J., Pemberton, J.M., Pilkington, J.G., Clutton-Brock, T.H., Coltman, D.W. & Kruuk, L.E.B. (2007) Quantitative genetics of growth and cryptic evolution of body size in an island population. *Evolutionary Ecology*, **21**, 337–356.

Wittum, T.E. & Perino, L.J. (1995) Passive immune status at postpartum hour 24 and long-term health and performance of calves. *American Journal of Veterinary Research*, **56**,

1149–1154.

Wojciechowski, W., Harris, D.P., Sprague, F., Mousseau, B., Makris, M., Kusser, K., Honjo, T., Mohrs, K., Mohrs, M., Randall, T. & Lund, F.E. (2009) Cytokine-producing effector B cells regulate type 2 immunity to *H. polygyrus*. *Immunity*, **30**, 421–433.

Wolak, M.E. (2012) Nadiv: an R package to create relatedness matrices for estimating non-additive genetic variances in animal models. *Methods in Ecology and Evolution*, **3**, 792–796.

Wolf, J.B., Brodie, E.D., Cheverud, J.M., Moore, A.J. & Wade, M.J. (1998) Evolutionary consequences of indirect genetic effects. *Trends in Ecology & Evolution*, **13**, 64–69.

Wolf, B.T., Howells, K., Nakielny, C., Haresign, W., Lewis, R.M., Davies, O. & Davies, M.H. (2008) Genetic parameters for strongyle and *Nematodirus* faecal egg counts in lambs and their relationships with performance traits. *Livestock Science*, **113**, 209–217.

Woolhouse, M.E. (1998) Patterns in parasite epidemiology: the peak shift. *Parasitology Today*, **14**, 428–434.

Wu, L.C. & Zarrin, A.A. (2014) The production and regulation of IgE by the immune system. *Nature Reviews Immunology*, **14**, 247–259.

Yang, J., Benyamin, B., McEvoy, B.P., Gordon, S., Henders, A.K., Nyholt, D.R., Madden, P.A., Heath, A.C., Martin, N.G., Montgomery, G.W., Goddard, M.E. & Visscher, P.M. (2010) Common SNPs explain a large proportion of the heritability for human height. *Nature Genetics*, **42**, 565–569.

Ye, C.J., Feng, T., Kwon, H.-K., Raj, T., Wilson, M.T., Asinovski, N., McCabe, C., Lee, M.H., Frohlich, I., Paik, H.I., Zaitlen, N., Hacohen, N., Stranger, B., De Jager, P., Mathis, D., Regev, A. & Benoist, C. (2014) Intersection of population variation and autoimmunity genetics in human T cell activation. *Science*, **345**, 1254665.

Zhang, X.K., Takashima, I. & Hashimoto, N. (1988) Role of maternal antibody in protection from hemorrhagic fever with renal syndrome virus infection in rats. *Archives of Virology*, **103**, 253–265.

Zuk, M. (1990) Reproductive strategies and disease susceptibility: an evolutionary viewpoint. *Parasitology Today*, **6**, 231–233.

Zuk, M. & McKean, K.A. (1996) Sex differences in parasite infections: patterns and processes. *International Journal for Parasitology*, **26**, 1009–1024.

Zuk, M. & Stoehr, A.M. (2002) Immune defense and host life history. *The American Naturalist*, **160**, S9-22.